

nature

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Too much British common sense

Ask a hardened British government watcher how he feels about the documents pushed at him, and his response will generally be equivocal, even though what he reads is often a model of clarity, coherence and common sense. The discussion paper *Future World Trends*, released last week by the Cabinet Office (HMSO, 60p), has, however, a big enough element of common sense to evoke profound worry, rather than mere ambivalence. For common sense is conservative; and the implicit no-change theme running through the content gives it an ostrich-like perspective.

The document is the product of an interdepartmental committee of (regrettably anonymous) government scientists chaired by Dr Robert Press of the Cabinet Office, and the committee's objective (apart from stimulating "informed discussion") was to make its own assessment of projections on population, food, energy, mineral resources and pollution and to "help establish the position to be taken by the United Kingdom in international matters".

Future World Trends is worrying for far more important reasons than the obvious one that the subject matter is gloomy. We knew that already. But, even allowing for its limited size and scope (and the immensely irritating lack of references), the product is below standard. Not only is it more interesting for what it leaves out, or refuses to emphasise, than for what it includes; the tone it adopts as a consequence also makes it far from suitable as a basis for genuine "informed discussion".

Amid all the interesting (if obvious) points made about exponential population growth, for example, the only real tilt made towards the now widely accepted view that high population growth is the consequence and not the cause of poverty is a mere mention of the rather woolly view endorsed at the 1974 World Population Conference in Bucharest that "voluntary reductions are primarily dependent on changing social attitudes which are more likely to come about as a result of improved living conditions". Nothing is ventured regarding the structural changes which might be necessary to allow just such an improvement.

On theoretical food needs early next century, the document says these could be met but are not in fact likely to be. But the crucial explanation is bland: there are "economic, social and political barriers to the optimal policy of concentrating the expansion of output"; "similar obstacles" restrict "equitable global distribution"; growth in the consumption of (inefficiently produced) animal protein foods in rich communities will continue. As with population, the implicit conclusion is not drawn out, namely, that there is something wrong with the global economy and its capacity to allocate resources effectively. The need for a "massive transfer" of resources to what it calls (wrongly) the "developing countries" is mentioned—but is then largely discounted.

The committee's discussion of mineral resources highlights very well the orientation which makes for its difficulties on such matters. The price mechanism, it explains, operates to bring supply and demand for mineral resources into equilibrium. Because of it, there are actually "no hard and fast physical limits to resources"; the limits are

"economic and technological". But the document (thoughts of OPEC?) adds, without elaboration: "national questions of environment and security of supplies and international constraints will undoubtedly affect the availability of raw materials". Later, on the broader use of nuclear energy, nothing is said of the proliferating international trade in nuclear technology which is its precondition. The prospect of nuclear blackmail, or indeed anything else which might upset present geopolitical arrangements and global economic patterns, is apparently not relevant.

What is relevant, it seems, is the 200th anniversary of Adam Smith. A look at the all-too-brief section headed "Economic aspects" highlights once again the ubiquitous price mechanism. This, we are told, will in general maximise welfare "subject to the distribution of income and wealth being socially and politically acceptable"; government intervention may be necessary in the event of "market failure". Yet it is precisely the unacceptability of the existing distribution which identifies the weakness of the market mechanism; and it is "market failure" (did it ever succeed?) which has spawned documents like this and its predecessors.

There is no reference to the idea that, without uninhibited expansion (which is what the debate is all about), and probably even with it, the market mechanism alone cannot produce anything other than an uneven allocation of resources; nor of the idea that there must be some sort of redistributing agency if the underdevelopment which is the corollary of the industrialised countries' development is to be forestalled. Yet the so-called "developing countries" (to the extent they are discernible as that—is Iran any longer comparable to Tanzania?), with few exceptions, are not developing at all; and the population and food problems will remain until real development takes place.

It seems incredible that a document looking at the next 30 years should evade all these points about the international economy and international politics. Scientists know that nowadays they will be judged for their public comments (or lack of them) regarding society as much as for their comments within their specialities—especially when they are participating in the gloom game. Yet this committee has largely failed to acknowledge that the real problems of the future will most likely be essentially political ones.

The necessary transfer of resources to ameliorate the population and food problems will demand sacrifices; so will the commitment of resources to solving these and other problems (energy, pollution). Such an awkward reassessment of priorities demands not only leadership but also authority—or, put another way, obedience. The sacrifice may therefore be, for the few countries which have elements of it, democracy itself. And it is a measure of the problem that it is democracy which offers the best chance that the consequence of an agonising choice might lead to success. The committee has chosen not to question the survival of the west's "business civilisation". Politics, after all, wasn't within its remit. So its ostrich-like perspective has contrived a camel. And a rather ordinary one at that.



An immunological approach

Barry Bloom, professor of microbiology at New York's Albert Einstein College of Medicine, looks at the possibilities for preventing parasitic diseases in cattle

WITH projections that world population will increase from 4,000 million to about 7,500 million by the end of the century, and an estimated 500 million people currently suffering from malnutrition, the magnitude of the world food crisis has finally touched the consciousness of people everywhere. Attention has been focused primarily on the green revolution and the need for increasing production of food grains; consequently the need for increasing production of meat and cattle has for the most part been overlooked.

The present situation is grim. While reliable statistics are virtually nonexistent, millions of head of cattle die of parasitic diseases each year, and more than 700 million are estimated to be at risk. In Africa alone, at least 7 million square kilometres of grazeable land, capable of supporting 120 million head of cattle, remain largely unproductive, chiefly because of trypanosomiasis and East Coast fever. The use of pesticides, while clearly important, has been inadequate to stave off these diseases, which are carried by the tsetse fly and ticks respectively. Effective drugs either do not exist or are too expensive for general use.

As a consequence of the enormous losses in potential food resources, there has recently been some discussion of the possibility of developing vaccines for immunizing cattle against diseases caused by blood parasites. In particular, the Rockefeller Foundation has sponsored a series of meetings to discuss the prospects.

Advocates of an immunological approach to control of parasitic diseases draw encouragement from two simple observations. First, if there were no immunity to such diseases, there would probably be no cattle in most of sub-Saharan Africa. Second, although mortality from these diseases is distressingly high, animals which survive infection are often resistant to subsequent reinfection. In fact, animals native to Africa, such as the n'dama, appear to be more resistant to parasitic diseases than species introduced from Europe. The general hope is that, with better understanding of the immune mechanisms required for protection, better characterisation of the parasite antigens required for resistance, and the use of immunological adjuvants—which are used remarkably infrequently in parasitic immunology—an immunological approach to control of these diseases could be realistic.

Although research has so far been limited, there have already been some promising results. Vaccines have been developed for protecting cattle against three parasitic diseases (babesiosis, anaplasmosis and East Coast fever) and immunisation has been found to offer protection against three others in laboratory animals (malaria, South American and African trypanosomiasis).

Even beyond our current wealth of ignorance of the specific aspects of host-parasite relationships, there exist serious problems which limit real progress in this area. A major problem is money. According to recent figures from the World Health Organisation, for example, in 25 African countries for which details are available, the average annual health budget is \$1.17 per capita, and in 8 countries it is less than \$1 per capita. In seven of those countries, the health budget is less than 5% of the total government budget. If those figures represent levels of support for control of human disease, the prospects for control of animal diseases by the developing countries themselves are clearly dismal. Moreover, the more developed countries hardly seem to be concerned with these problems—in recent years, there has been a general reduction in bilateral and international assistance programs in health-related areas.

A further problem is the increasing criticism of meat production and consumption in the West. While this campaign has relevance to grain-intensive cattle production in the developed countries, there is a danger that it may inhibit development of important food resources in developing nations where grain-intensive feeding is neither necessary nor possible. In sub-Saharan Africa, for example, cattle feed on low grade brush and natural vegetation, on lands generally too poor to support cultivated crops.

Promising developments

In spite of those problems, however, there have been some promising developments against the following widespread parasitic diseases.

● The most enigmatic and biologically fascinating of the animal parasite diseases is East Coast fever, caused by *Theileria parvum*, a virtually unclassifiable tick-borne parasite. The disease, which takes the form of a galloping proliferation of lymphocytes, not unlike leukemia, is responsible for the death of half a million cattle a year.

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The extraordinary aspect of the disease in cattle is that the parasite grows only in lymphocytes, and appears to act in a manner similar to cancer-causing viruses in transforming lymphocytes into continuously growing cell lines.

There has been progress in producing immunity in cattle in two ways: by infecting them with ticks, followed by antibiotic treatment, and more recently, by immunising them with infected lymphocyte cultures. The hope is that the parasite can be isolated from infected lymphocyte cultures, and the protective antigens purified and characterised.

● A million young cattle die each year from another tick-borne disease, anaplasmosis. It is caused by a family of rickettsiae which infect and multiply within mature erythrocytes. An attenuated vaccine has been produced by subjecting the parasite to gamma radiation and passing it repeatedly through an unnatural host, the sheep. Striking protection against tick infection has been observed in small scale experimental trials in the United States, and more extensive field trials are under way in Mexico and Columbia.

● A third tick-borne disease, babesiosis, is responsible for the death of about a quarter of a million cattle a year. The parasite is related to the malaria parasite, and similarly multiplies in and destroys erythrocytes. An attenuated organism has been produced by passing the parasite through splenectomised cattle, and a highly effective vaccine, currently in use in Australia, has been developed.

● African trypanosomiasis, the most serious of the animal diseases, is responsible for the death of 3 million cattle a year. The animal disease and human sleeping sickness, both of which are caused by parasites carried by the tsetse fly, are clinically very similar. They represent a very difficult immunological problem because the parasite

has developed a highly effective method for eluding the immune system of the host, known as 'antigenic variation'. The disease is characterised by waves of illness, reflecting rapid growth of the parasite, followed by remission engendered by an immune response, followed by sequential emergence of antigenically different organisms.

Because the organism has not yet been grown in culture, it is not known by what genetic or regulatory mechanism it manages to change its surface characteristics to evade the immune system. Two different lines of research for potential vaccination are, however, being followed. The first would involve modifying the immune system of the host so that it would react quickly to wipe out the second appearance of the organism before it reaches sufficient numbers to produce disease. Following the recent isolation of the major antigenic surface molecule, the second would involve the production of a "cocktail" vaccine containing a mixture of antigens from the most common variants to combat the different forms of the organism. Both require better characterisation of the organism and its antigens.

● There are many similarities between the animal and human parasitic diseases, such as Chagas disease and malaria, and there are consequently common research problems and strat-

egies. Chagas disease, caused by the organism *Trypanosoma cruzi*, affects 30 million people, chiefly in Latin America. Ten per cent of its victims, virtually all children, die from an acute disease, while the rest develop a chronic illness with invasion and destruction of heart and smooth muscle. It frequently ends in sudden death. Although there is no effective treatment at present, an irradiated vaccine has been produced from parasites grown in animal cell cultures. It provides virtually 100% protection to massive challenge in susceptible mice, which are killed by a single virulent trypanosome.

● Two hundred and fifty million people now suffer from malaria worldwide, and at least a million people die from the disease in Africa alone. Two successful types of experimental vaccines against different forms of the organism have been produced, but practical limitations on the quantity of these vaccines preclude either of them from being useful on a large scale. One line of research is aimed at defining the nature of the protective antigens so that synthetic antigens could be developed as potential vaccine material.

There are major scientific questions to be understood and problems to be overcome that will require contributions by molecular biologists and geneticists as well as parasitologists and

immunologists, and at this stage even if increased support for research were forthcoming, it would be foolish to guarantee that inexpensive and effective vaccines and drugs would result. Even if some were to be developed, there would remain two further serious problems. Although effective vaccines, particularly against African trypanosomiasis, would offer the possibility of opening vast areas in the tsetse belt to cattle production, without thoughtful assessment of the ecological consequences and intelligent regulation, marginal areas of the Sahel and tropical Africa could be destroyed by overgrazing. The second problem is the lack of an effective health infrastructure in the developing countries. There is a critical need for developing health services and training adequate health manpower to provide basic information and care for the health needs of the human and animal populations.

At the very least, it is worth considering what could be accomplished simply by supporting studies on the epidemiology of the animal parasitic diseases. It is an activity which could involve large numbers of untrained young people in an important scientific and educational enterprise. It would serve to develop a pool of talent from which the best could be selected for more advanced training in the health sciences and services. □

USA

Behind the politics of influenza

The idea of the United States government footing the bill to prevent the American population falling sick makes President Ford's request for funds to attempt a mass inoculation against flu something more than a medical issue in this election year. From Washington, Colin Norman gives the background

ON FEBRUARY 3, a young army recruit stationed at Fort Dix, New Jersey, came down with what seemed like a typical case of influenza. But the next day, while on a training march, he collapsed and died. His death sparked off an extraordinary series of events, culminating last week in the launching of the most extensive and controversial mass inoculation campaign ever undertaken in the United States.

The cause of the soldier's death turned out to be pneumonia, but a virus isolated from his respiratory tract immediately set alarm bells ringing in public health laboratories around the world. It was found to be a strain of

influenza virus, commonly found in swine, which had not been involved in human infections for nearly 50 years. The virus, moreover, is very similar to the strain which caused the most serious flu pandemic in modern history, the 1918-19 outbreak which swept around the world killing some 20 million people.

Though only 12 other cases of swine flu were subsequently confirmed at Fort Dix, a massive monitoring effort on the base found antibodies to the flu strain in the blood of some 500 recruits, suggesting that they had been infected with swine flu during the outbreak. If the young recruit hadn't died, the outbreak would simply have been put down to the so-called Victoria/A strain which has been widespread in the United States this winter, but the isolation of the swine virus may have provided advance warning of a possible new flu pandemic next winter.

Because swine flu hasn't been abroad in the human population since the late 1930s, nobody under the age of 50 has any antibodies to guard against it. And

it is likely that even those who do have antibodies are not protected against the disease. Thus, if the Fort Dix outbreak is not just a freak, isolated event, the virus could spread like wildfire through an unprotected population. Dr Edwin Kilbourne, a leading flu researcher at Mount Sinai School of Medicine in New York, said last week, for example, that "our thought is that probably the virus is seeded now in foci around the country, and when winter comes we will see it again".

For several weeks after the virus was isolated and identified, committees of scientists and government officials met at the US government's Center for Disease Control in Atlanta, Georgia, to decide what, if anything, should be done to counter the potential threat. Last week, they decided to recommend a massive inoculation effort against the virus, and President Ford appeared at a press conference, flanked by two of the most well known scientists in the United States—Jonas Salk and Albert Sabin—to announce that he is requesting \$135 million from Congress for a crash programme to inoculate every man, woman and child in the United States by the end of November.

The decision was immediately controversial. Officials from the World Health Organisation expressed surprise, some scientists argued that since there's no definite evidence that the Fort Dix outbreak has spread there is no need for a massive inoculation campaign, and there have even been dark hints that the whole thing is an election-year ploy to attract votes. Nevertheless, it can reasonably be argued that Mr Ford had little choice in the matter.

One-jump-ahead virus

The influenza A virus is an amazing organism, which has managed to evade man's attempts to keep it in check for three decades. Its ability to cause epidemics lies in its extraordinary capacity to stay one jump ahead of the body's natural defence mechanisms by changing its genetic makeup every few years. (Two other classes of influenza virus, designated B and C, are rather less elusive, however.) When a person is infected by an influenza virus he develops antibodies which will give immunity from subsequent reinfection, but if the virus undergoes a slight genetic change, the antibodies often won't recognise it and the virus can slip past the body's defences and cause disease. The flu virus's ability to change its character is also the reason why attempts to develop vaccines against flu have been relatively disappointing—vaccines developed against one year's flu virus are often ineffective against a modified strain which may appear the following year.

Aside from those small elusive changes, the influenza virus is also capable of major genetic shifts. Every so often, a radically new variation of influenza virus emerges and, because there is no natural resistance to it at all, it can cause major pandemics. The last such pandemic occurred in 1968

from a strain first isolated in Hong Kong (hence its popular designation as Hong Kong Flu); another worldwide epidemic occurred in 1957 from a strain which first appeared in Asia.

If, as seems to happen, pandemics appear in roughly 10-year intervals, a radically new flu strain can be anticipated in the late 1970s. The Fort Dix swine flu virus may be that strain. If so, it would be the first time that the world has had advance warning of a pandemic, and that warning could provide time for preventive action. But the evidence is far from conclusive; hence the controversy over Mr Ford's decision to launch a mass inoculation campaign.

Following the isolation of swine flu virus at Fort Dix, a massive study was conducted to see whether the virus is showing up anywhere else in the United States. No other cases were found, and the virus also seems to have disappeared at the army camp. It is therefore possible that the outbreak was a freak event which suddenly ended through unknown causes. But, on the other hand, it is possible that the virus has been seeded in the population and will re-emerge during next winter's flu season.

Kilbourne notes, for example, that "historically we have never recognised a new (flu virus) variant without a major pandemic following in its wake", and he added that the Asian and Hong Kong flu epidemics "smouldered before they took off" in the United States. Dr Robert B. Crouch, professor of infectious diseases at Baylor College of Medicine, is also not surprised that the Fort Dix outbreak seems to have disappeared. He pointed out that the Asian flu epidemic was first spotted in the United States as an isolated outbreak in Iowa which died off, followed by another isolated outbreak in Pennsylvania, which also disappeared; two months later, the full-fledged epidemic began to appear.

Time barrier

The problem now, however, is that there's not enough time to wait and see whether other outbreaks of swine flu occur before taking evasive action. Production of the 200 million flu shots which will be required to inoculate the entire US population must begin immediately or the programme won't be completed before next winter. Thus Ford asked Congress last week for funds to begin manufacturing vaccines from the Fort Dix strain as soon as possible, with the goal of inoculating high risk people (the elderly and infirm) by late summer, and the rest of the population by November.

Some scientists have argued, however, that even if the virus does reappear next winter, there is no need

to mount a major campaign against it now. Dr Sidney Wolfe, head of Ralph Nader's Health Research Group, argues, for example, that the analogies which have been drawn between the Fort Dix strain and the swine flu virus which caused the 1918 pandemic have been greatly overstated. The huge death toll from the 1918 outbreak resulted from secondary infections such as pneumonia, which can now be cured by antibiotics, and so there's no reason to suppose that a new flu pandemic would be as devastating. It should be pointed out, however, that the Hong Kong flu pandemic killed some 20,000 people in the United States and cost more than \$3,000 million in terms of lost work and medical expenses, antibiotics notwithstanding.

One particularly disturbing, but inevitable, factor is that the vaccine now being developed will only be effective against the Fort Dix swine flu strain, but the Victoria/A strain and an influenza B strain are also expected to reappear next year. People will therefore be receiving flu shots but can still expect to get a variety of influenza, a fact which won't increase public confidence in medical science.

A more alarming possibility, which cannot be dismissed out of hand, is that an entirely new flu strain could emerge in the southern hemisphere during the next few months as the harbinger of a different flu pandemic. In that case, people in the United States could be getting immunized against the wrong pandemic strain.

Be that as it may, President Ford was presented with a problem for which there is really only one solution. Though the evidence is sketchy, it is possible that a pandemic flu strain has been isolated in time to take precautionary action. Mr Ford could gamble that the virus won't reappear and do nothing, in which case he would bear a heavy responsibility if the virus did show up again, or he could ask Congress for \$135 million as an insurance policy.

Though those involved in the decision vehemently deny that politics played any part, it cannot be denied that if Ford refused to initiate an inoculation program and swine flu became rampant in October, the effect on the November elections could be decisive. By the same token, it is unlikely that Congress will deny the request for the funds.

● A statement released last week by Britain's Department of Health in response to developments in the United States said that on present evidence its advisory group on influenza would not be recommending vaccination. The group suggests, however, that it would be a wise precaution to incorporate the new virus into anti-flu vaccine.



President Ford : requesting \$135 million

BRITAIN

ALMOST two years after it was officially adopted as a run-up to Britain's fast breeder reactor programme, the Steam Generating Heavy Water Reactor (SGHWR) made the headlines again last week with a report that a starting date has at last been set for the construction of the first commercial installation at Sizewell in Suffolk.

Even in passing that historic milestone, however, the two organisations with major development interests in the new reactor were still displaying the sort of confusion and uncertainty that has dogged the project's progress since it was first mooted at the beginning of the decade. While the Department of Energy readily confirmed that the first sod will be turned in late 1977, both the Nuclear Power Company (NPC), responsible for producing the reactors planned for Sizewell and Torness, and its main customer, the Central Electricity Generating Board (CEGB), denied that a firm date had been set.

If not cagey, NPC is at least determined not to tread on sensitive CEGB toes, seeming eager to stress only that virtually every figure ever mentioned in connection with the project has little more solid basis than the paper it is written on. NPC says that reports putting the price of the first two SGHWRs (totalling 4,000 MW) at up to £1,500 millions cannot be taken seriously as they have not outlined the separate elements contributing to the total sum or considered whether they are capital or recurring costs. With so little being said, it is an understandable omission.

The CEGB's sensitivity is, nonetheless, forgivable in view of the fiasco that has accompanied the development of the ill-fated Advanced Gas Cooled Reactors (AGRs). Tight CEGB lips last week parted just wide enough to explain that a nominal sum spent at the drawing board could later save millions on site modifications—a lesson expensively learned with the Dungeness B AGR. So, whatever the Department of Energy may proclaim, there will be no firm starting date from CEGB or NPC until final design details have been submitted by NPC, accepted by the CEGB, and passed by the Nuclear Installations Inspectorate.

● The academic year 1974-5 will not be remembered as a great year for universities; but at least they all stayed open. That, roughly, is the conclusion of the University Grants Committee's (UGC) report for last year published recently (Cmnd 6435, 50p). Supplementation, in bits and

pieces, of the basic grant could not soften entirely the cut in real terms of about 8% in the recurrent grant that UGC handed on to universities.

Many of the economies that were made in that year are not repeatable, or indeed maintainable for much longer. 500 academic posts were frozen. The consequences, the lucidly-



written report points out, are "a staff distribution growing increasingly at variance with student distribution; lack of new appointments [for] promising young graduates, and failure to develop new areas of study which should be their province". Mobility is being blocked as tenured staff cling to their posts.

Building programmes, apart from those to bring medical student numbers up to 4,100 by 1979 and those to house computers, are almost at a standstill. Nothing was done for libraries, twelve of which already have proposals for expansion and another ten of which are expected to look for expansion soon. More radical proposals to alleviate library problems are likely soon.

One trend which took UGC by surprise was that a number of universities want to start new courses in food science and technology, and nutrition. The UGC wasn't convinced that there was that much need, but it might do a bit more enquiring, as those in food seem to say the opposite.

A disturbing aside to the report was that students are now arriving at universities well-motivated, with good paper qualifications but lacking some of the basic skills. Modern languages, science and technology seem to be the worst sufferers, and remedial courses are taking up valuable university time. The reasons are complex, no doubt; the lack of well-qualified school teachers, failure of universities to respond to curriculum changes in schools, too much froth and not

enough staple diet in some school courses—these are all likely contributors. The Education and Science Minister himself appealed to school heads just last week to try and boost by a tenth the numbers of students with A level passes in science and maths going to universities.

● Discouraging news for the UK fishing industry. In spite of the British Trawler Federation's pressures, which have already led Foreign Secretary James Callaghan to protest in Brussels about the inadequacy of the 12-mile national fishing limit called for by the EEC Commission, it has become clear that the industry is not going to get the 100-mile limit it wants.

Facing the Select Committee on Secondary European Legislation last week Mr Callaghan's junior Minister, Roy Hattersley, left no room for doubt that UK negotiations with her European partners would not be aimed at achieving such a broad exclusive zone within the proposed 200-mile community strip. Neither Mr Hattersley nor the junior minister at Agriculture, Fisheries and Food, Ted Bishop, felt compelled to comment on speculation that the UK will press for a 35-mile national limit.

What is certain is that Britain will not use her veto to reject the Commission's proposals. Mr Hattersley told the committee that the real problem was rather whether her partners could be dissuaded from using their vetoes to block British pressure for an adapted Common Fisheries Policy. The British view is that adaptation is crucial because, while apparently hamstrung over exclusive limits by obligations under the Treaty of Rome, her industry feels that it will lose more than other EEC members if the Commission's call for national quotas based on past catches is adopted. The practical uncertainties surrounding the effective policing of an eventual agreement add to the complications.

● The Secretary of State for Energy, Anthony Wedgwood Benn, who last week withdrew from the battle for the Labour Party leadership, is continuing his fight to introduce a long-term energy strategy for Britain. He now intends to approach the issues in Britain's energy policy on a wider front, and plans are under way for a meeting in June or July, when it is hoped that several major international oil companies will line up with management and union representatives from the newly-formed British National Oil Corporation and the other nationalised energy industries.

INDIA

Becoming well oiled

India is increasing its investment in oil, and the aim is self-sufficiency. Our correspondent in Jullundur reports on a vital aspect of the sub-continent's energy equation

FOR over two years now, India has been groaning under the weight of her mounting oil-import bill. From around 3,000 million rupees a year before the 1973 Arab-Israeli war it is now running close to 12,000 million rupees—accounting for a third of her entire export earnings and a quarter of the total value of her imports. This is despite the fact that the Government, through a number of fiscal and other measures taken in early 1974, has managed to limit consumption and to curb the rate of growth of demand for oil. These measures remain in force.

Consumption rose in 1975 by only 2.7%, from 21.74 million tonnes to 22.34 million tonnes, the 1974 figure being substantially lower than an estimated 25 million tonnes calculated on the basis of an earlier average annual growth rate of about 9%. Consumption of petrol during 1975, at 1.25 million tonnes, was slightly lower than the 1974 figure of 1.28 million tonnes, despite a considerable increase in the number of powered vehicles in the country; the items contributing to increased consumption of petroleum (and products) included naphtha (14.5%), kerosene (5.5%), high-speed diesel (4.7%), furnace oil (0.5%) and cooking gas (14.3%). The increase in the offtake of naphtha was largely due to better capacity utilisation by fertiliser manufacturing units, while good rains helped dampen demand for diesel for agricultural purposes which was running some 12% higher during the first six months of 1975 compared to the same period in 1974.

India has been meeting only about a third of her oil requirements (including related products) from indigenous sources—mainly onshore wells in the states of Assam and Gujarat. She has had to import the rest from various oil-exporting Arab countries and Iran through bilateral agreements. The Union Petroleum and Chemicals Minister, Shri K. D. Malaviya, recently told the Parliamentary Consultative Committee attached to his Ministry that, because of India's adverse balance-of-payments and foreign exchange positions, the situation regarding imported oil was likely to be very difficult during 1976. Neither was it known how and when OPEC's oil facility for developing countries would be set up, nor when results would start flowing from the

ongoing "north-south" conference. The country expected to produce 9.5 million tonnes of oil this year, Shri Malaviya told the committee, and his ministry was making all possible efforts to ensure an import of 14 million tonnes from external sources to meet the country's essential requirements. [It has since been reported that arrangements have now been made for the import of a major part of the crude oil India needs from other countries].

India has passed through a very difficult period but, while she is still not completely out of the woods, pressure seems to have eased somewhat due to an all-round improvement on the energy front. Among other things, coal production has increased enough to wipe out scarcities, and power generation is presently pretty close to meeting all requirements so that power cuts imposed in most states have been withdrawn. While much of the improvement has come through better management and better performance by both man and machine, good and plentiful rains during 1975 have played no mean part in transforming the picture.

For all the havoc it has wrought on the country's economy, the oil crisis has also done a few good things: the Oil and Natural Gas Commission (ONGC) has been stirred into vigorous action and its redoubled efforts to find more oil onshore and offshore are beginning to yield results. From onshore fields in Assam and Gujarat, production this year is expected to be more than 8.2 million tonnes compared to 7.5 million last year. In three to four years' time, according to the Petroleum and Chemicals Minister, the figure will go up to 11 million tonnes.

Production could commence shortly from India's first offshore wells in the

Bombay High region. Two developmental wells, along with other installations including submarine pipelines to take oil to the shore, are nearing completion. Production will start at the rate of 2 million tonnes a year by the end of 1976, and will gradually be stepped up to 10 million tonnes by the end of the decade. While the ONGC explores and exploits the Bombay basin, two foreign firms are working on the Bengal and Kutch basins. The very first well drilled in the Bay of Bengal was reported to be oil bearing, raising hopes of further breakthroughs. [The Minister recently announced an oil discovery of "very great significance" at the Bassein structure off the western coast].

In 1974, the ONGC acquired three new vessels, two for drilling in the Bombay High region and a third for seismic surveys in the continental shelf near Madras. The Petroleum Ministry has also set up a "deeper continental shelf" project to explore the 20,000 km region west and north-west of the Bombay High area. Various steps are also being taken to make the country self-sufficient in the manufacture of all types of equipment needed for onshore and offshore exploration and production of oil. The first country-made rig is expected to be available in about two years' time. A variety of oil-field equipment is already being manufactured in India, and its production is being stepped up.

India has also entered into agreements with Iran and Iraq to look for oil in these countries. According to the Petroleum Minister, some 750,000 tonnes of Rustum crude from India's exploration venture in Iran will be brought to India this year.

Before the turn of the century, India is expected to become self-sufficient in crude oil. Some optimists think she could even become an exporter of oil by the year 2000. □

Science and religion

If circulation figures of periodicals and journals are any indication, religious subjects continue to score over scientific ones in popularity among the literate population in India, which has the world's third largest scientific and technological community. According to a recent study, there were 927 scientific and technical journals (all languages) compared to 1,221 religious and philosophical ones in the country by the end of 1973, and circulations of the latter went up by more than the former over the decade 1963–1973.

Broken down by language, the figures for the circulation of Bengali,

Tamil, Kannada and Gujarati science periodicals showed a downward trend during the decade, whereas those of the religious journals in these languages went up over the same period. Marathi, the language of the state of Maharashtra, had the unique distinction of having more readers of scientific journals than those of the religious ones. Urdu was found to be another language with a distinction—it showed an increase in readership of scientific journals with a simultaneous decrease in circulation of the religious periodicals. In English and Hindi—the two languages with the largest proportion of periodicals on all subjects—readers seemed to prefer religion to science.

IN BRIEF

Group of Thirteen?

The seven nuclear powers who recently agreed terms extending safeguards against the misuse of exported nuclear technology are expected to be joined in their pact by six more nations, reportedly after Soviet pressure to have more eastern bloc countries admitted. Belgium, Czechoslovakia, East Germany, Poland, Sweden and the Netherlands would participate with Britain, Canada, France, Japan, the USSR, the USA and West Germany in an agreement which allows nuclear trade with states outside the Non-Proliferation Treaty. The report follows news that France is planning to sell nuclear reactors to Libya, a major

oil producer facing none of the problems of energy shortage.

LST refocus

The US House of Representatives last week took the first step toward rescuing the Large Space Telescope (LST) from acute budgetary failure, by passing a bill which authorises the expenditure of \$3 million on planning and instrument design for the mission. At the same time, however, the Senate Aeronautical and Space Science Committee approved a bill which, in tune with the Ford Administration's own decision for the coming fiscal year, contains no funds at all for the LST. But the LST seems very likely to get

full backing next year.

Shtern support

The case of Dr Mikhail Shtern, the Ukrainian endocrinologist at present serving an 8-year-sentence in a labour camp near Khar'kov, continues to evoke support. On March 24 a petition was published in Paris, signed by 51 Nobel Prize winners, accusing the Soviet government of fabricating charges against him and demanding his release. Ironically, the same day, Dr Shtern, who had made an appeal for clemency on the prompting of the camp authorities that it would be favourably received, was informed that it had, in fact, been rejected.

THE year 1976 is the hundredth anniversary of the passing of the Cruelty to Animals Act, which still controls the way in which painful animal experimentation — "vivisection" — is performed in Britain. There is now considerable pressure to introduce new legislation, partly because of a general feeling that a law made over a hundred years ago must, in some ways, be out of date. Animal experiments have indeed changed during that period. In 1876 only a few hundred experiments were performed each year, and a high proportion involved surgery. Today we make some five million experiments per annum; the majority are to test drugs, pesticides, food additives, cosmetics and other chemicals, and consist of feeding trials which may involve little of the type of "pain" with which the original Act was concerned.

The 1876 Act is unpopular with lawyers, perhaps because it has given rise to so little litigation. It would be cynical to suggest that the lawyers' criticism stems from the fact that so few fees have been earned in this field; their more legitimate complaint is that the absence of decisions by the courts may leave the interpretation of some of the Act's provisions ambiguous. However, the exhaustive deliberations of the Royal Commission set up in 1906, and contained in their report which eventually appeared in 1912, suggested that radical changes were not required. The very thorough report, in 1965, by the Home Office's Departmental Committee on Experiments on Animals (the "Littlewood" report) came roughly to the same conclusion. The Act may not be technically perfect, but it works better than many newer pieces of legislation. Once more there is much to be said for leaving well alone, though the Littlewood proposal for a

more effective Advisory Committee, containing lay members, would seem worth implementing without further delay, notwithstanding the apparent opposition of the Home Office.

In my experience most responsible experimenters have a strong feeling that certain potentially painful experi-

vances from the regions where ethical controls do not exist. In fact progress has generally been greatest in the laboratories where controls are most strictly enforced.

Some anti-vivisectionists insist that animals are so different from man that experiments on animals are generally inapplicable to human conditions. It is true that, in the end, new drugs and treatments must be tried out on man himself, but without preliminary animal experiments on several species most physicians would be even more reluctant than at present to make these trials. More experiments on man would probably mean doing more, not fewer, animal experiments. In fact there are already many instances where "human guinea pigs", healthy volunteers, are usefully employed, though in a civilised country, except for the often-gruesome experiments done by some scientists on their own bodies, this type of work has been considerably restricted. It is sometimes suggested that important medical problems could be rapidly solved if humans were used without any ethical constraints. This I doubt. We have the experience gained from experiments on prisoners in Nazi concentration camps. Here humanitarian concern was seldom allowed to modify the plans of the scientists, and millions of victims were killed in the name of research. Yet no major discoveries were made. It seems that the brutal scientist is generally a bad scientist, and it matters little whether he works with animals or with man.

If we are to have new legislation, research workers as a whole would welcome it if it only curbed the excesses of the minority of bad and cruel workers, but there is a danger that it might make much productive and valuable work, done under good ethical conditions, impossible.

A hundred years on**KENNETH MELLANBY**

ments are seldom if ever justified. This is an ethical judgement, but it may have a scientific basis. Some countries have no regulations to prevent any cruel experiment, and even some states in the USA allow practices outlawed elsewhere. Thus we read of school children inserting electrodes in monkey's brains, and I recently reviewed an American book in which children were instructed how to subject rats and other mammals to noise intensities sufficient to destroy their auditory tissues. It is sometimes argued that the controls imposed by the 1876 Act hold up medical and scientific progress. If this were so, then we would expect striking ad-

correspondence

International transfers

SIR,—The following document is communicated for publication on behalf of the 52 Fellows of the Royal Society whose signatures appear below. No doubt other members of the scientific community in this country and throughout the world will wish to affirm their support for the same principles. Those who share our concern about these matters may wish to know that a Study Group on Scholarly Freedom and Human Rights has been established by the Council for Science and Society (3/4 St Andrews Hill, London EC4V 5BY) to give further consideration to the issues involved. It would be particularly valuable to the work of the Study Group to have further detailed information or well informed opinion from any of your readers.

Yours faithfully,

J. M. ZIMAN

and M. F. ATIYAH, C. H. ANDREWES, C. AUERBACH, J. F. ADAMS, J. G. BOLTON, J. BADDILEY, K. G. BUDDEN, C. A. CLARKE, S. CURRAN, R. E. DAVIES, F. DANTON, C. D. DARLINGTON, K. G. DENBIGH, L. ESSEN, C. A. FLEMING, G. FRYER, G. HERZBERG, W. HUDSON, A. HADDOW (deceased), J. T. HOUGHTON, J. H. HUMPHREY, W. K. HAYMAN, A. KLUG, A. KELLY, H. KORNBERG, O. A. KERENSKY, B. LOCKSPEISER, L. F. LA COUR, H. LIPSON, J. D. MCGEE, E. H. MANSFIELD, J. MANDELSTAM, K. MAHLER, A. NEUBERGER, J. NEEDHAM, L. E. ORGEL, A. G. OGSTON, M. R. POLLOCK, J. W. PRINGLE, W. S. PEART, G. ROCHESTER, A. ROBERTSON, R. A. RAPHAEL, N. SHEPPARD, G. SERIES, M. SEATON, M. SWANN, M. SZWARC, R. WHITAM, M. WILKINS, F. YOUNG.

The following statement is proposed for consideration and adoption by learned societies, academies, governmental bodies and private corporations involved in the practical arrangements for the international transfer of scientific information by written communication and by personal travel. Although not intended to be legally binding, this statement indicates the basic principles by which we consider that particular agreements and actions in this sphere ought to be judged.

International Scientific Communication

The claim of scientific knowledge to universal validity can only be tested and maintained by international intercourse between scientists. All nations which make use of scientific knowledge benefit by the rapid diffusion of information about new discoveries, and by the submission of new ideas to the widest circle of competent critics. The free exchange of knowledge and ideas is fundamental to the health and vitality of science as an enterprise of all mankind.

The individuals and corporate bodies who benefit directly from this intercourse

should be bound by certain common courtesies and mutual obligations. Although these courtesies and obligations are normally taken for granted, their neglect or violation can cause much ill will and damage to science as a whole. We therefore believe that there is value in stating the following general principles, which are based upon the experience of communities in which science has flourished in many different historical and political circumstances.

A. Written Communications

1. The published literature of science should be free to circulate, without hindrance or censorship, within and between nations.
2. All efforts should be made to ensure that relevant scientific information published in any one country be made available, as rapidly as possible, in the most convenient form, to scientists and to scientific institutions in all other countries.
3. The originality of published scientific work should be respected throughout the world by fair practices of citation or other attribution.
4. The legal rights of authors and publishers of scientific journals and books should be protected under the International Copyright Agreement.
5. All national scientific communities should contribute to the financial support and management of international facilities for the transfer of scientific information such as abstracting and indexing services.

B. Individual Travel

1. Scientists acknowledged by their colleagues as experts should be free to travel within and between different countries for the exchange of unclassified scientific information.
2. Acknowledged scientific experts making such visits should be given intellectual hospitality in the form of access to open research institutions and to individual scholars.
3. The services of scientific experts from all countries should be made available to assist in the activities of the international scientific agencies.
4. Bilateral financial and administrative arrangements for the exchange of scientists between two countries should not be regarded as excluding or limiting visits arranged by other means.

C. Conferences

1. The appropriate national scientific organisations should be encouraged to participate in open international scientific conferences by facilitating the attendance of well-qualified scientific experts in the relevant subjects.
2. All countries should endeavour to contribute to the material facilities for international scientific conferences by way of hospitality and financial support.
3. Every bona fide participant in an international scientific conference should be accorded freedom of entry to the meeting place of that conference.
4. The expert authority of properly constituted international committees of scientists should be respected in such matters as the choice of participants and speakers at international scientific conferences.

Genetics at the OU

SIR,—The article by Professor Steven Rose on the new genetics course offered to Open University students (*Nature*, 259, 437; 1976) is misleading. In particular, it does not refer to the existence of a Consultative Committee, of which we are members, appointed by the Nuffield Foundation to advise on the development of the course, and to the sharp disagreement between that committee and Professor Rose about the suitability of the materials now being distributed for use by students at large but also by Open University students.

The Joint University Genetics Course was launched in 1973 with a grant of £20,000 from the Nuffield Foundation with the objective not merely of developing an outstanding course in genetics but also of proving a mechanism by which the Open University and other universities might collaborate in the development of educational materials. At the same time the Foundation appointed a Consultative Committee to advise the course team on academic matters and to advise the Foundation on the progress of the work. Apart from members of the course team, the committee included Professor D. Lewis (University College, London) as chairman of the committee, Professor W. Bodmer (University of Oxford), Professor J. Jinks (University of Birmingham), Professor D. Jones (University of Hull), Dr H. Kacser (University of Edinburgh), Professor R. H. Pritchard (University of Leicester) and Professor J. Sang (University of Sussex). In March 1974 the total amount of the grant from the Nuffield Foundation was increased to £88,000.

At the end of 1975, nearly three months before Professor Rose's article appeared, the committee informed the Nuffield Foundation that it was dissatisfied with the outcome of the previous three years' work and asked that the course should not be published, or even used by Open University students, without further consideration, restructuring and amendment. The Nuffield Foundation has informed the Open University of the committee's discontent but no agreement has yet been reached between the Foundation and the University.

The length and depth of the course was thought to be far beyond the capacities of the intended students. The Open University states that the study

time for the average "unit" is 12 hours. Furthermore, these 12 hours must be completed, on the average, in one week since the course is a half-credit. This, for a part-time student, means a minimum of two hours daily study after normal work for the whole of the academic year. It is also a *second* level half-course. It was the committee's considered opinion that this is an impossible task if understanding at a university level is aimed at. In their experience, the course, as it stands, would be a demanding one for full-time students having no second half-credit to cope with and enjoying much greater academic support than Open University students. The committee suggested that the university should cut the course in half or offer two alternative (half-) courses.

In spite of the original remit to write a course which is suitable for other than Open University students, the Consultative Committee has not been presented with any evidence that this problem has been seriously considered. One of the principal claims for obtaining support from the Nuffield Foundation was that it was not a purely Open University exercise but that other institutions would benefit equally. It is extremely doubtful if this objective is in any way near realisation. (One of the obvious deficiencies of the course soon to be offered for general sale is that the practical manual that students at universities other than the Open University will need has not been published, or even written.)

The Consultative Committee jointly with the Nuffield Foundation suggested a number of changes which would have gone some way to meet these criticisms, but they were not accepted by Professor Rose.

It is not appropriate to comment at length about the many doubts and criticisms which the committee had both about the nature of some of the units and the manner in which the course was produced. Suffice it to say that it is the opinion of the committee that the adherence to a timetable, set down before the magnitude of the problem was appreciated, made it almost mandatory that no fundamental thinking could inform the writing of the course. We have sadly come to the conclusion that a great opportunity has been lost in making use of the generous provision of the Nuffield Foundation.

Yours faithfully,

H. KACSER

Department of Genetics,
University of Edinburgh

R. H. PRITCHARD

School of Biological Sciences,
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J. SANO

School of Biological Sciences,
University of Sussex, UK

Birds on the Chagos Bank

SIR,—The Great Chagos Bank, 6°15'S, 72°E is all that remains of a once gigantic atoll. The dry land now consists of eight small islands set on the rim of the atoll and an outlying group of six other islands collectively called Egmont. Together they constitute a terrestrial habitat of only 828 ha, of which Eagle and Egmont Islands make up 80%.

Records indicate that at some time between 1813 and 1937 all the islands came under the influence of the copra industry. Only Nelson island was left in its "natural" state which included colonies of nesting sea birds, the eggs of which were collected to supplement the diet of the copra workers on the nearby Salomon group.

Recent surveys show that:

- on all the islands broad-leaved forest is beginning to replace the coconut plantations;
- on all islands other than Eagle and Egmont there are large populations of nesting sea-birds, numbering in total more than 100,000 pairs—dominantly terns and boobies, but including 15 species in all;
- on Eagle and Egmont there are feral brown rats and only a few hundred birds; the vegetation is, however, similar to that of the other islands.

In order to realise the full potential of the island group to nesting seabirds we suggest the removal of the rats from Egmont and Eagle Islands using a combination of methods such as "warfarin" and Liverpool virus. The islands are remote from any large centre of population and are rarely visited. Such action could allow the already overcrowded bird colonies on the local islands to expand and new species to move in from further afield. For instance, pairs of Abbots Booby were seen in the vicinity of the islands on two occasions. This is the rarest of the Indian Ocean's gannets, whose only contemporary nest site on Christmas Island is threatened by development and ecological change.

We estimate that if such action were successful the total nesting population of birds in the area could rise to in excess of 0.5 million pairs.

We are planning, subject to legislative approval, to attempt to exterminate the rats on Egmont and Eagle Islands in early 1977. We invite comment on the desirability and feasibility of this course of action.

Yours faithfully,

M. J. HIRONS

College of Education,
Easthampstead Park, Berkshire

D. J. BELLAMY

C. SHEPPARD

Department of Botany,
University of Durham, UK

Bugs Bunny

SIR,—Schedule 1 of the Conservation of Wild Creatures and Plants Act 1975, passed recently by the UK Parliament, provides the best way of giving full protection to any animal whose survival is threatened. To be included an animal must be given a common and a scientific name ("Naming the Loch Ness Monster", *Nature*, **258**, 406; 1975). Thus, after ten years of searching, we would like to present the evidence on which we base our description and naming of the "Model E Rabbit".

The best photograph shows the head of the rabbit obscuring the DNA bands of an analytical caesium chloride gradient (below). This gradient was run in a Beckman Model E Analytical Ultracentrifuge (Wells, R., and Ingle, J., *Pl. Physiol., Lancaster*, **46**, 178–182; 1970), and the rabbit must have obscured the light path while the plate was exposed.

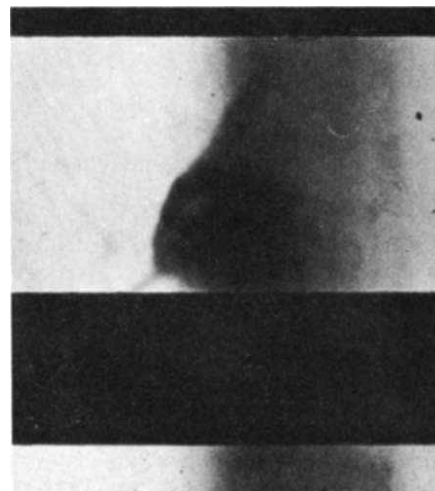
We feel that previous failure to observe these rabbits, for surely there must be a breeding population, is due to their shy and retiring nature; the DNA on this occasion was from cucumber hypocotyls and their inordinate fondness for these may have led to this rash exposure.

In the interest of protecting this rare and endangered species we have decided to name it *Oryctolagus barbi-fugenter* because of the curious and distinctive beard-like structure characteristic of the species and because the animal so readily flees from view. It has been pointed out to us that this binomial is an anagram of "Centrifuge laboratory bugs", but this is thought to be entirely coincidental. We hope for the support of the World Wildlife Fund in our efforts to preserve and study this unique rodent.

Yours faithfully,

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L. POTTS

Department of Botany,
University of Edinburgh,
Edinburgh, UK



news and views

How far does pollen travel?

from Peter D. Moore

POLLEN grains contain the male gametophyte generation of flowering plants. They are rendered mobile by such forces as wind, water or insect vectors, and the distances which they travel determine to a large extent the degree of gene movement between populations of a species. It is therefore of considerable interest to a geneticist to establish how far pollen of a particular species is dispersed. Such a knowledge is also of value to the palaeoecologist, who is concerned with the interpretation of fossil assemblages of pollen grains in terms of the vegetation which gave rise to them; reconstruction of past vegetation can be considerably aided by information regarding the dispersal potential of different species.

The distances moved by pollen grains depend on their density and aerodynamic properties, which vary from one species to another. Erdtman (*Handbook of Palynology*, Munksgaard, Copenhagen; 1969) has estimated the settling velocities of various types of pollen grains from the air and has obtained such values as 3 cm s^{-1} for *Pinus*, 1.6 cm s^{-1} for *Alnus* and 0.9 cm s^{-1} for *Juniperus*. These three species are wind pollinated and one might suppose that a low settling velocity will be accompanied by a high proportion of the grains produced by a plant being transported over long distances. Precise correlation studies are complicated by the fact that some species produce far more pollen per individual than do others. The local surroundings of the pollen-producing plant will also have an important influence both upon the amount of pollen produced and upon the distance which it is likely to travel. The hazel, *Corylus avellana* for example, flowers far more abundantly on forest edges and in clearings than it does when enclosed by a woodland canopy. Tauber (*Proc. Congr. Int. Assoc. Quatern. Res.*, 7, 131; 1967) has also demonstrated that plants surrounding the pollen-producing individual act as a filter. Pollen grains become impacted upon solid obstacles in just the way that snow adheres to

tree-trunks and hence their dispersal potential is reduced. This filter acts in a differential manner, for large grains are more easily impacted than small ones. The height above the ground at which the pollen is released will also influence the distance over which it is likely to remain airborne.

In the case of wind-pollinated species, the atmospheric turbulence patterns are also of crucial importance in determining dispersal and Tauber (*Danm. geol. Unders. IIR* 89, 1; 1965) has devised a detailed model of pollen transport above and below woodland canopies, which takes into account the low wind speeds found within tree canopies and the slightly raised wind speeds within the trunk space. Some canopy-produced pollen will be incorporated into air masses moving at higher speeds above the woodland shelter and this may be carried considerable distances. Tyldesley (*New Phytol.*, 72, 175; 1973) has recorded the pollen of trees, mainly birch and pine, on the Shetland Islands in densities of up to 30 grains m^{-3} of air. The movement of air masses before collection suggests that these could have been transported from Scandinavia. Similarly, Moar (*New Zealand J. Bot.*, 7, 424; 1969) has reported the pollen of *Casuarina* in surface samples from New Zealand, when the nearest plants of this genus grow in Australia. On the basis of aerial transport studies on pollen in Canada, Christie and Ritchie (*Naturaliste Canad.*, 96, 531; 1969) come to the conclusion that the mean life of pollen grains in the troposphere is about three days.

It is difficult to assess the importance of such long-distance pollen transport mechanisms to the process of gene flow. Since only a very small proportion of pollen grains produced is likely to be carried very far, one assumes that these grains are of little functional significance. Yet Mason (in *Genetics, Palaeontology and Evolution* (edit. by Jepsen, Simpson and Mehr), 356, New York; 1963) records the invasion of *Pinus muricata* genes into populations of

P. remorata on the islands of Santa Cruz, over 30 miles offshore. This must have come about as a result of pollen movement from the mainland, hence long-distance pollen transport can be an important vehicle for gene movement.

The study of pollen movement has been rendered difficult by the problem of relating pollen grains, once dispersed, to the producer individual. The process of gene movement itself has proved a useful indicator of pollen dispersion, for example in the studies of rye grass, *Lolium perenne*, by Griffiths (*J. Agric. Sci., Camb.*, 40, 19; 1950). Such studies have shown that wind-pollinated species have a pollen dispersion pattern which is leptokurtic; this distribution pattern resembles the normal distribution, but frequencies fall more rapidly on either side of the mode (see Proctor and Yeo, *The Pollination of Flowers*, 262, Collins, London; 1973). Wright (*J. Forestry*, 51, 114; 1953) has expressed the dispersal capacity of certain trees in terms of the standard deviation of their leptokurtic pollen curves.

The use of radioactive labels on pollen grains has been attempted. Colwell (*Am. J. Bot.*, 38, 511; 1951) tagged the pollen of *Pinus coulteri* with ^{32}P and collected dispersed pollen in open Petri dishes for counting. He showed that the bulk of the pollen released was deposited within 10 m. Chamberlain and Chadwick (*Ann. appl. Biol.*, 71, 141; 1972) have applied tracer techniques to the study of pollen impaction on solid objects. The usefulness of such techniques for the study of pollen dispersal in the field, however, is limited by the environmental hazards involved.

In this issue of *Nature* (page 422), Handel describes the use of neutron activation analysis in an experiment to determine the extent of pollen dispersal from two species of *Carex* in a woodland habitat. A solution of samarium oxide was injected into male spikelets and the day after pollen was shed, female spikelets from the same and

from neighbouring plants were collected. When samarium is bombarded with neutrons it is converted into europium, ^{153}Eu , which emits gamma rays which can be counted. In this way, samarium-containing pollen can be located accurately. The results obtained obey the characteristic leptokurtic distribution, and show that very little pollen of either species tested is carried beyond 1 m, although the species with the taller culm (20 cm as opposed to 12 cm) does have better powers of dispersal. The very small distance travelled by the pollen of these understorey herbs confirms what has been assumed about pollen movement beneath tree canopies. It also suggests that there must be a limited flow of genetic material between isolated individuals or

populations of such species in woodlands, even if they produce copious pollen, depend upon wind pollination and have a tall culm.

The technique of neutron activation analysis in pollen studies offers considerable opportunities. The field is now open for the study of pollen movement from canopy species, the precise influence on dispersal of impaction on tree trunks, pollen dispersal from clearings and woodland edges and also the movement of pollen in insect-pollinated species. The technique, given the basic equipment, is fast and accurate and lacks the environmental hazards associated with radioactive labels. We can now await a flow of new data which will be of value to geneticist and palaeoecologist alike. □

Hominid remains from Hadar, Ethiopia

from our Palaeoanthropology Correspondent

ALTHOUGH fossil-bearing deposits in the Hadar region of eastern Ethiopia have been explored only since 1973 it is already clear that this area is of extraordinary importance. As two recent papers indicate (*Nature*, **260**, 289, 293; 1976), extremely favourable depositional conditions have allowed the preservation of many fossil specimens, even crocodile and turtle eggs, in a virtually complete state. This is an almost unique situation for Plio-Pleistocene deposits in Africa. Moreover, appropriate geological materials are available for chronometric dating of the sequences and although the absolute dates obtained so far are not entirely satisfactory, this problem should soon be resolved. Available information indicates that the hominid sites in the Hadar area are between 2 and 4 million years.

As reported in last week's issue, fossil material from approximately 17 hominids has been recovered from sites in the Hadar region. Of these, one of the most significant is AL 288, a partial skeleton recovered from the younger levels of the sequence. The partial skeleton is of a very small hominid which bears many similarities to the australopithecines from Sterkfontein, in South Africa. Certainly one of the most interesting aspects of this individual, from information available so far, is the direct indication of limb proportions in this early Pleistocene

hominid. Estimates of upper and lower limb proportions have been made previously but never on virtually complete bones from a single individual. The ratio of humeral/femoral length in AL 288 is 83.9; living *Homo sapiens* has a mean of approximately 73. By comparison, among the living great apes *Pan*=102, *Gorilla*=116, *Hylobates*=113 and *Pongo*=135. (Schultz, *Hum. Biol.*, **9**, 1937). Thus, although the length ratio of AL 288 is somewhat above the human mean it is distinctly lower than that for the living great apes. Such a relationship has been expected, especially in view of the long humerus from East Rudolf (Leakey, Mungai and Walker, *Am. J. Phys. Anthropol.*, **36**, 1972) and the long ulna from the Omo River area just to the west of Hadar (Howell and Wood, *Nature*, **249**, 174; 1974). These data clearly indicate the essential man-like body conformation of AL 288. This is of particular interest in view of Oxnard's recent claim (*Nature*, **258**, 389; 1975) of the orang-like morphology of the australopithecines. This new information on limb proportions, along with the clear evidence of erect bipedality in the morphology of the pelvis, femur and knee joint does not lend support to such an assertion. Moreover, it indicates that computer-oriented analyses on single bones, however sophisticated the statistics, may be of somewhat limited value in determin-

ing morphological and functional affinities of fossil material. This is especially true when, as Oxnard recognised, the functional pattern of the fossil specimen may have no modern parallel.

Of additional interest in these papers is the report of two palates (AL 199 and AL 200) which Johanson and Taieb claim resemble other material referred to as *Homo erectus*. This material is from the lowest stratigraphic unit known to date in the Hadar region; if the data on the Kadada Moumou basalt is confirmed then this material would pre-date 3 million years. This possible early occurrence of *Homo erectus* is of particular interest since the re-dating of the KBS tuff in the East Rudolf area (Curtis *et al.*, *Nature*, **258**, 395; 1975) would suggest that the early members of the genus *Homo* in that area are little more than 2 myr old.

Results of field work in the past few years have made it clear that at least two forms of hominid co-existed in east and possibly South Africa in Plio-Pleistocene times: one, a less advanced form usually called *Australopithecus* (or *Paranthropus*) and a more advanced form, *Homo*. This picture has achieved additional confirmation from the new material recovered at Hadar. The complexity of the fossil evidence at East Rudolf has led Richard Leakey to suggest a possible total of four hominid lineages there (*Nature*, **248**, 653; 1974) and Johanson and Taieb, in the present paper, suggest possibly three at Hadar. Whatever the total number of contemporaneous hominid taxa in this area, it is clear that we are not seeing a simple and orderly progression of ancestor-descendant populations. The most economical interpretation of this diversity is that the early hominid line, once it had come to occupy the forest-margin/savannah ecozones, experienced a number of radiations and adaptations to this habitat. Such adaptive radiations are common during the initial occupation of a new habitat zone and serve to fit the immigrant populations to the available new niches. The molecular evolutionists, who have postulated a relatively recent origin for the family Hominidae, have made palaeontologists somewhat reluctant to talk about a great time depth for the hominids. But that is exactly what this Plio-Pleistocene diversity indicates. Such diversity, with two or more lineages, clearly suggests considerable antiquity for the human line, with splits having occurred in different places, at different times and in response to different environmental situations. Perhaps the appropriate question, however, in the face of such apparent diversity is not how many hominid lineages have existed but how many hominid niches are available in such a habitat? □

Sociobiology: a new synthesis and an old quarrel

from Robert M. May

One of the most notable books published last year was *Sociobiology: The New Synthesis* (Belknap, Cambridge, Massachusetts; 1975), by Edward O. Wilson. This massive work aims to draw together a growing body of field and laboratory observations on animal behaviour, and combine it with insights drawn from population biology and ecology, to yield an understanding of how different social systems have evolved. Sociobiology is Wilson's term for this systematic quest for an evolutionary explanation for all forms of social behaviour. Both explicitly and implicitly the book calls attention to some of the major unsolved problems: this is indeed Wilson's main aim, which would be described more accurately if the title read *Towards a New Synthesis* rather than *The New Synthesis*.

Evolution of social systems

The fundamental principle guiding this sociobiological enterprise is that an organism's evolutionary success is measured by the extent to which its genes are represented in the next generation. One road to such success is for the individual to evolve characteristics (strength, speed, camouflage, a well advertised nasty taste or the misleading mimicry of something else's advertisement) which help it survive long enough to reproduce; these relatively straightforward considerations have provided the focus for most investigations of evolutionary ecology. For many animals there are, however, advantages (such as greater foraging efficiency, or defence against predators) in living groups. Once this happens, the social structure of the community, and the behaviour of individuals within it, themselves become subject to selection. Social systems thus evolve to maximise the genetic fitness of individuals in specific environments.

If the social system is sufficiently close-knit, an individual may enhance its genetic contribution to the next generation by the apparently altruistic act of sacrificing itself in order to help its kin. An extreme example is provided by the sterile worker castes in insect societies; here recent work by Hamilton and by Trivers and Hare has produced quantitative insight into the organisation and evolution of the social insects (see *Nature*, **260**, 9; 1976).

The behaviour of birds and mammals is less stereotyped than that of insects, and elucidating the evolutionary basis

of their social structure is correspondingly a more complicated and ambiguous task. Nevertheless, with the growing tendency towards long-term, analytical field studies of individual animals whose consanguinities and social histories are recorded, much progress is being made.

Consider, for example, the studies by Schaller, Bertram and others on the Serengeti lion (for example Bertram, *Sci. Amer.*, **232**, (5), 54-65; 1975). Roughly speaking, the typical female lion spends her life as a member of the pride into which she is born, and may live about 18 years. In contrast, male lions leave or are ejected from the pride at puberty, and they will usually enjoy a later brief tenure of 2-3 years, typically along with one or more brothers, as the only adult males in another pride: the more brothers sharing the rule, the longer the tenure. It seems that, soon after taking over a pride, the new males kill the resident cubs. Since the females tend not to produce a new litter until the previous cubs are 20-30 months old (which time is of the same order as the males' expected tenure as owners of the pride), and since the new males usually share no genes with the pre-existing cubs, the males' inaugural act of infanticide is adaptive from their point of view, although it is certainly not in the females' best interest.

This is a particularly brutal example of the tensions between male and female interests which arise in diploid systems when parental care is important, as it is among birds and mammals. A female's genes are represented directly only in the offspring she herself produces, and so it tends to be in her interest to care for them (at least until they begin to be able to look after themselves, whereupon at "weaning" a parent-offspring tension arises because it is in the child's interest to continue to enjoy parental care, whereas the mother will do better to turn attention to producing a new litter). A male's genes have a chance of direct representation in the progeny of every female he inseminates, and in some circumstances his genetic input into the next generation will be maximised by striving for copulations with many females (even at the expense of evolving otherwise maladaptive ornaments, and at the risk of injury in fights with other males), and giving no help in raising the children. In short, in many situations there is a built-in tendency for females to focus on ecological re-

sources such as food and nesting sites, and for males to focus on females.

Mating systems

These notions, when combined with the ecological details of particular environments, help explain the bewildering diversity of mating systems observed in bird and mammal societies: monogamy; polygamy (either as polygyny, one male with several wives, or as polyandry, one female with several husbands); promiscuity. In some situations raising offspring is necessarily a two-person job, leading to monogamy. Monogamy is the rule among birds (92% of all bird species are monogamous), and is uncommon among mammals, where the mother suckles the young. In environments where resources are very unevenly distributed, it may pay a male to devote much energy to defending a good territory, and it may pay a female to share that territory with other wives rather than have exclusive partnership with a male who owns inferior real estate; this leads to polygyny. Polyandry should be very rare, and it is. When the female is capable of feeding both herself and her offspring, as is the case for most herbivorous mammals, we expect a tendency towards promiscuous mating systems. But carnivorous mothers need help: all raptorial birds are monogamous, and carnivorous mammals tend to have complicated mating systems (such as the lions, above).

When the environmental circumstances dictate that a male should opt for monogamy, and devote his time and energy to help rear offspring, it will be adaptive for him to react violently to the possibility of stolen copulations with his wife. In one series of experiments, Barash (University of Washington) placed a model of a male mountain bluebird near the nest, during the breeding season, while the real male bluebird was temporarily absent: on its return, the male fiercely attacked both the model and its own mate, in one case even driving her away and taking a new mate. Once the eggs had been laid, the male was much less traumatised by this mean trick, and did not attack its mate. This experiment, incidentally, shows how the manipulative techniques that shed so much light on the adaptive significance of animal behaviour cannot blithely be applied when dealing with humans.

Sociobiology summarises many other aspects of animal behaviour and social

organisation that can be illuminated by evolutionary thinking. A crisp review of some of this work is due to Kolata (*Science*, **187**, 50–51 and 156–157; 1975). When one comes to the primates (excluding man), social structures become very complicated and diverse, ranging from troops with a complex social organisation (baboons), through nuclear families (white-handed gibbons, with a single mated pair accompanied by up to four immature offspring and an occasional aged male), to individuals who spend most of their lives in solitary fashion (male orangutans).

So far I have touched on 25 of the 27 chapters in *Sociobiology*, which deal with non-human animals. This work constitutes a *tour de force* of synoptic scholarship, and has not been challenged.

Human behaviour

In chapters 1 and 27, Wilson speculates upon the insights to be gained by applying sociobiological thinking to human behaviour and human societies.

The social insects, for example, show how extreme forms of altruism can evolve by virtue of kin selection in conjunction with inbreeding. In the absence of any constraints, there is a strong selective advantage in inbreeding coupled with recognition and co-operation among kin. But in relatively complex organisms, such as humans, there is a severe constraint, namely the well documented increase in the expression of deleterious genetic traits as heterozygosity is diminished by inbreeding. Wilson poses the question: how are the gains through inclusive fitness and losses through inbreeding depression balanced? In which life history strategies is this balance struck so as to increase the likelihood of more intense inbreeding and social life? In particular, he observes that in human societies (excepting Faulkner's Yoknapatawpha County) the incest taboo is both universal and has the merit of minimising the occurrence of crippling genetic malformations. He suggests that this universal taboo has adaptive origins, rooted in some gene complex.

In suggesting that pervasive human behavioural manifestations such as spite, indoctrinability or courage may have aspects that are genetically controlled, Wilson is at pains to emphasise that he is not implying "that there exists one gene for spite, another for homosexuality, and so on, as one might envisage the inheritance of flower colour or seed texture in garden peas. The tendency to develop such behaviour, in a distinctively human form, is part of an immensely complex social repertory which is undoubtedly dependent on large complexes of genes" (*Harvard Gazette*, January 16, 1976). Given that the relatively simple and stereotyped behaviour of aggregation in slime moulds is under the control of a complex of about 50 genes (Cox, Princeton University, personal communication), I find Wilson's view plausible. It is, however, clear that direct confirmation, in the form of explicitly identifying particular gene complexes for particular behaviour, is out of the question.

These first and last chapters, with their suggestion that human behaviour

ONE long-standing objective in polymer science is the production of fibres with an elastic modulus (ratio of stress to strain) approaching the theoretical maximum value. It seems self-evident that the structure should consist of polymer chains aligned as closely as possible to the fibre axis with few folds or disoriented regions. Various methods might produce this structure. The natural and most elegant method is exemplified in the production of plant cellulose where polymerisation occurs *in situ* so that fibres grow by addition of monomer units to one end. This has never really been achieved synthetically.

The drawing process used in the production of textile fibres produces chain orientation by stretching the fibre to several times its initial length. Two fibre industry alumni now in universities have worked on modifying this process to produce high draw ratios and orientation and so high moduli. E. S. Clark of the University of Tennessee and co-workers at Du Pont have produced acetal fibres with a modulus of 35 GPa (1 giga pascal = 10^9 N m⁻²) (*Polymer Engng Sci.*, **14**, 682; 1974). For comparison, carbon fibre has a modulus of 500 GPa and the theoretical maximum modulus of polyethylene is 220 GPa (Frank, *Proc. R. Soc. Lond.*, **A319**, 127; 1970). These acetal fibres were drawn at about 130 °C by a factor of 20 times in a two-stage process. The usual room temperature draw ratio is 7 times. The structure seems to consist of a single highly oriented phase.

I. M. Ward and co-workers at Leeds have studied the dependence of draw ratio on temperature, molecular weight and pre-draw microstructure in polyethylene. They found that the modulus increases roughly linearly to 70 GPa at a draw ratio of 30 times. The draw ratio was a maximum for a sample of high molecular weight and narrow molecular weight distribution crystallised by cooling to 110 °C then quenching to room

Strong fibres

from Paul Calvert

temperature and drawn at 75 °C (*Polymer*, **15**, 233; 1974; *Nature phys. Sci.*, **243**, 143; 1973). This leaves the question as to whether it is the molecular weight itself or its influence on the microstructure that is important in allowing a high draw ratio.

Barham and Keller (*J. Materials Sci.*, **11**, 27; 1976) have taken up this point and looked at the relationship between modulus, draw ratio and starting structure. They find that the dependence of modulus on draw ratio is the same for a range of pre-draw microstructures. What did change with microstructure was the maximum draw ratio which could be attained. Sheet which had been directly quenched or made from a sample with no low molecular weight polymer could not be drawn past 20–25 times as opposed to 30–35 times. This point is somewhat confused in

the paper by the fact that the figure captions are exchanged.

From this Barham and Keller deduce that the low molecular weight chains, which segregate to the spherulite boundaries during slow crystallisation, govern the drawability of the sample. In support of this they say that samples which draw well had a black line around the spherulites when viewed with crossed polars. Presumably this region is liquid at the drawing temperatures and somehow allows large deformations without fracture. This is surprising in that one would expect strong rather than weak boundaries to be necessary.

Finally, mention should be made of the high melting point, high modulus fibres produced by spinning aromatic polyamides and polyhydrazides from strongly hydrogen bonding solvents such as dimethylacetamide–lithium chloride or sulphuric acid in which they form lyotropic liquid crystals. These fibres spun from anisotropic solutions have moduli several times those of fibres spun from similar isotropic solutions and reach 140 GPa. They are reviewed by Carter and Schenk (*Structure and properties of oriented polymers*, edit. by M. Ward), where the industrial significance is evidenced by the fact that most of the important references are to the patent literature. Two fibres, called Kevlar and Kevlar 49 by Dupont, which are based on poly (p-phenylene terephthalamide) look likely to be used in tyre belting and in composites for body armour and aircraft parts.

may have some components which are genetically determined, have aroused some people's wrath. To suggest for example, that the evolutionary considerations which determine the mating systems of mammals and birds have any light, no matter how oblique, to shed on the tensions and asymmetries commonly observed in human sexual relationships is to invite reflexive dismissal as a "sexist".

A fairly typical example of this criticism is a letter in the *New York Review of Books* (November 13, 1975), written by a group of students and professors in the Boston area, in reply to Waddington's favourable review of the book. This intemperate letter begins by putting the book squarely in a historical tradition which runs from the Social Darwinism of Herbert Spencer, through the gas chambers of Nazi Germany, to E. O. Wilson. It continues with a series of selected and re-arranged quotations which form a collector's item for connoisseurs of polemical literature. The substance of this and other criticisms is that by suggesting a genetic component to human behaviour, Wilson is advocating "biological determinism": they fear that in the hands of political demagogues this can be bent to a defence of the *status quo* as the "natural", and therefore correct order of things.

Broadly speaking, the controversy revives the hoary nature-nurture debate. Wilson is suggesting that human behaviour has innate components, and that there are limits to its plasticity, whereas his critics seem to see man as standing apart from the rest of the animal kingdom, being unique in that human behaviour and social organisation have no discernable genetic elements.

Contrary to the tenor of the criticism, nowhere does Wilson say that human behaviour is totally determined by the genes. He emphasises that there is much about human behaviour that is indeed unique, the product of man's intelligence and social institutions. In an interview with the *New York Times* (November 9, 1975); he said "I see maybe 10% of human behaviour as genetic and 90% environmental. Lewontin [a population geneticist at Harvard who is prominent among the critics] would see it as zero % genetic and all environmental".

This, in turn does violence to the critics' position, which is more subtle. As I see it, their position is rather that until genes for behaviour are specifically identified, the discussion is less than definitive; this being so, and since Wilson's speculations can be perverted to evil political ends, they warrant attack. In Lewontin's words, "At present our ignorance on this question is so enormous, our investigatory tech-

niques so primitive and weak, our theoretical concepts so unformed, that it is unimaginable to me that lasting, serious, truths about human nature are possible. On the other hand the need for the socially powerful to exonerate their institutions of responsibility for the problems they have created is extremely strong. Under these circumstances any investigations into the genetic control of human behaviors is bound to produce a pseudo-science that will inevitably be misused." (*Harvard Gazette*, January 16, 1976).

Wilson's reply in the same *Gazette* is that "We should be especially cautious about closing off an entire area of investigation when it is in its most vulnerable exploratory stage, because of a generalised fear about its possible eventual misuses in political life. Human sociobiology is in that early stage. I realise that its current hypotheses and facts are susceptible to perversion; equally by ideologues on the right or the left, I might add. We should discourage the perversion—but not the subject."

Shifting from defence to attack, Wilson argues that insofar as human

behaviour may contain genetic aspects, social programmes which deny this fact do so to their disadvantage. "[Human] evolution took place under conditions which in many cases no longer exist; our moral precepts, and the economic and social systems that flow from them, will have to be directed to the problems of the future... One does not efficiently eliminate injustice stemming from a genetically based human nature by denying the existence of that nature or prohibiting research on it." He stresses that his views on human sociobiology should not hinder legislation towards social reform, any more than the genetic nature of near-sightedness should discourage people from getting glasses.

The attack on Wilson's book seems to me to have been conducted with a certain incivility, and lack of attention to what the book actually says. When human sociobiology finally comes of age, I hope it will explain why people who are moved to passionate concern for the well-being of Mankind (be it in small disputes such as this, or in grand revolutionary or religious movements) behave so harshly to individual examples of the species. □

Disturbing the radiation belts

from Kenneth G. Budden

It has been known for many years that electromagnetic waves from the Earth's surface can enter the magnetosphere and return to Earth. The earliest known and perhaps the most striking example is the phenomenon of whistlers. These were first observed in the nineteenth century (Preece, *Nature*, **49**, 554; 1894) but were not satisfactorily explained until 1953 (Storey, *Phil. Trans. R. Soc. Lond.*, **A 246**, 113). A whistler originates as the electric impulse from a lightning flash and much of its energy is in the audio frequency range. It travels up through the ionosphere into the magnetosphere and is guided along the lines of force of the Earth's magnetic field, so that it returns to Earth near the magnetically conjugate point in the other hemisphere. During its journey through the magnetoplasma it is dispersed in time so that the high frequencies arrive first. Thus, when received on an aerial connected to an audio amplifier, it is heard as a whistle falling in pitch. Later work showed that signals from other terrestrial sources could travel through the magnetosphere in this "whistler mode". Signals of frequency 15.5 kHz from the US Navy's transmitter NSS at Annapolis, for example, were received at Cape Horn (Helliwell and Gehrels, *Proc. Inst. Radio Eng.*, **46**, 785; 1958) both by the whistler path in the mag-

netosphere and by the more usual path in the space between the Earth's surface and the ionosphere, which at these frequencies is like a waveguide.

In the magnetospheric plasma these audio frequency whistler waves can only travel in directions making small angles, less than about 19°, with the Earth's magnetic field. This was once thought to be the guiding mechanism but it is not enough to account for the observations. It is now believed that these signals travel in ducts of enhanced ionisation aligned along the magnetic field lines. The signals are confined in the ducts in much the same way as the waves inside a waveguide. But even this is not enough to explain the great strength of some whistler signals. Whistlers have been observed to travel back and forth between the northern and southern hemispheres as many as twenty or more times, and sometimes they get stronger as they travel suggesting that the magnetosphere must be acting as an amplifier.

Since about 1958 it has been known that there are trapped energetic particles in the magnetosphere—the Van Allen radiation belts. These are mainly electrons and protons with energies in a wide range from 10 keV or less to 50 MeV or more, which travel in bent helical paths round the lines of force of the Earth's magnetic field. At the

latitudes where the lines of force come down towards the Earth and converge the particles are turned back or "mirrored". Thus they are trapped in stable helical paths with repeated mirroring in alternate hemispheres. A line of force of the Earth's magnetic field is described by its distance from the Earth's centre at the highest point, that is, where it crosses the equator. This distance is written LR_0 where R_0 is the Earth's radius, and we speak of the L value of a line of force. The radiation belts extend up to about $L=7$ or 8 with two maxima of intensity around $L \sim 1\frac{1}{2}$ and $L \sim 3\frac{1}{2}$. In between there is a minimum or "slot", in the range $2 < L < 3$, which was shown in Van Allen's first map of the radiation belts (Van Allen and Frank, *Nature*, **183**, 430; 1959).

The particles of the radiation belts can sometimes generate electromagnetic waves of audio frequency. These travel in the whistler mode to the ground and are observed as the remarkable rising and falling whistles that Storey (*op cit.*) called the 'dawn chorus'. The particles can give amplification of whistler waves, operating in a similar way to a travelling wave tube amplifier and this is the amplification needed to explain the observed strengths of some whistlers. Emission of radiation from the particles can be triggered by electromagnetic waves and triggering by whistlers and by signals from radio transmitters of very low frequency has been known for many years. Triggering by the harmonics of the 60 Hz electric power system of North America has been observed more recently (Helliwell, Katsufakis, Bell and Raghuram, *J. geophys. Res.*, **80**, 4249; 1975). Observations were made simultaneously at Siple in Antarctica and at Robertval, Quebec which is near the aluminium smelting plant at Arvida which uses roughly 1,000 MW at 60 Hz. The rectifiers generate high harmonics, up to about the 90th, that is about 5 kHz, which enter the magnetosphere and trigger emission at other frequencies. The triggering is believed to occur mainly over the equator. The triggered emissions travel to Earth in the whistler mode and can be observed at Siple.

Electromagnetic waves can, in turn, influence the particles of radiation belts so as to change their orbits. One possibility is that the pitch angle of a particle's path is decreased, so that it is made to move more nearly parallel to its guiding line of force. This means that its helical path is more open and it can penetrate further down towards the ground. Then, for many of the particles, mirroring does not occur. They are "precipitated" into the Earth's lower atmosphere, and the radiation belts are correspondingly depleted. Dungey (*Planet. Space Sci.*, **11**,

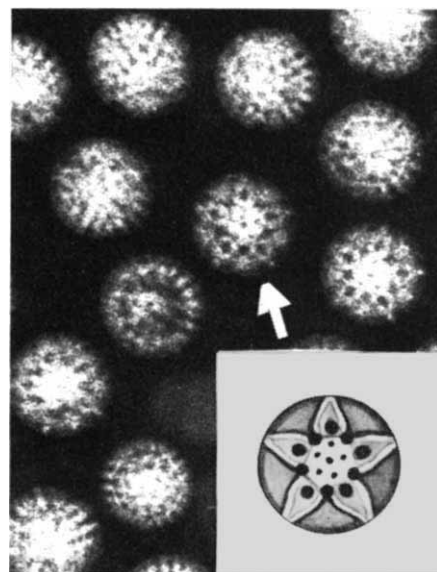
591; 1963) suggested that electromagnetic signals from the Earth might modify the radiation belts in this way, and there was speculation as to whether the electron slot in the range $2 < L < 3$ could be explained by this mechanism.

A recent study of emissions, mainly in the audio frequency range, made with the satellites Ariel 3 and 4, has greatly strengthened this possibility (see Bullough, Tatnall and Denby, page 401 in this week's issue of *Nature*). The analysis shows that harmonics of the 60 Hz power system in North America might contribute appreciably to the precipitation of particles. VLF radio transmissions from NAA (Maine, 17.8 kHz) and GBR (Rugby, 16 kHz) may also play a part. But thunderstorms in the American and African continents deliver a very large amount of electromagnetic energy into the magnetosphere, and further observations and analysis will be needed before we can be certain that the man-made sources of energy are really significant. □

The importance of being rotavirus

from June D. Almeida and
Arie J. Zuckerman

LATELY there has been considerable interest in the characterisation by direct electron microscopy of viruses in faeces (see *Nature*, **254**, 557; 1975). Recent publications report reovirus-like agents in stools (Kapikian *et al.*, *Science*, **185**, 1049; 1974), infantile gastroenteritis virus or IGV (Newman *et al.*, *Nature*, **258**, 631; 1975), duovirus (Bishop *et al.*, *Lancet*, **ii**, 1281; 1973), rotavirus and orbivirus (Flewett *et al.*, *J. clin. Path.*, **27**, 603; 1974). To the uninitiated it might seem that there has been a vast increase in the number of virus types to be identified in acute gastroenteritis. Closer examination reveals, however, that each of these descriptions concerns the same virus, which is now associated with acute diarrhoea in the human infant, the calf, the mouse, the pig and the foal. The outstanding feature of this virus is its morphology and at least three of the terms suggested are based on this morphology. Duovirus refers to the double-shell construction, orbivirus to its overall circular appearance and rotavirus refers to the spoke-like outer projections which give it a distinctive wheel-like appearance. It now seems that under certain circumstances the same virus can reveal a distinctive star-like pattern which is shown in the figure and interpreted in the inset diagram. The structure illustrated was



A group of rotavirus particles from a calf faecal homogenate. The majority show the morphology associated with this virus type and both complete and incomplete forms are present. In addition there are two particles showing the star arrangement described. $\times 225,000$. Inset, diagrammatic representation of the stellate particles based on the detail present in a number of them. To be compared with the arrowed particle in the main micrograph.

obtained from a specimen of faeces from a calf with diarrhoea. The titre of the particles was particularly high, estimated as being in the order of 10^{11} particles ml^{-1} . Rotaviruses consist of two concentric capsids each built up from distinctive, geometrically arranged subunits. The star-like morphology demonstrated may be a superimposition pattern formed by the interaction of these two structures. On the other hand, it may be that the stellate form shown in the figure is the intact form of this virus, and the ability to distinguish two components implies that breakdown has occurred.

This finding suggests that the situation could be further confused by the name astrovirus, one already used for gastroenteritis viruses by Madeley and Cosgrove (*Lancet*, **ii**, 451; 1975) or even stellavirus. But rather than introduce yet another name for this already terminologically overlaid group it seems better to fit this new morphological finding to an existing term. The diagrammatic inset emphasises a certain similarity between the new morphological feature of this virus group and a mediaeval "wheel of fortune". Thus two separate aspects of this virus group exhibit a wheel-like appearance, in turn suggesting that the term rotavirus may well be the most appropriate for this group which seems to move in wide circles throughout the animal kingdom. □

THE main obstacle to progress in understanding polarity and morphogenesis in embryos is the elusiveness of morphogens. One of the latest to confront the problem is Bruce Alberts, working in Lewis Wolpert's laboratory at the Middlesex Hospital Medical School on sabbatical leave from Princeton and DNA replication. He is attempting to isolate the morphogen from the polarising zone of the chick limb bud—an approach which Wolpert, concluding the Symposium, compared to a direct assault on Everest without oxygen. Wolpert belongs to a school which, feeling ill-equipped to scale the heights, has elected to tackle the foothills by constructing physically plausible theories to account for morphogenetic phenomena at the cell and tissue level—and thus elucidate the principles of morphogenesis rather than try to elucidate its molecular mechanism.

The predictive power of one such theoretical construct has been tested by Harold MacWilliams (Albert Einstein College of Medicine, New York), in an experiment aimed at distinguishing between Wolpert's theory of polarity and morphogenesis in *Hydra*, and that of Alfred Gierer and Hans Meinhardt (University of Tübingen).

Wolpert postulates two gradients, one activating and the other inhibitory, both with a source at the head end. The Gierer–Meinhardt model is more complex. It too is based on gradients, giving short-range activation and long-range inhibition of head formation; but it involves a further gradient, in activator source density. In the concrete metaphor used by MacWilliams, source density could be the density of cells synthesising activator, and the short-range activation gradient the concentration of free activator. The pattern of these gradients re-establishes itself after experimental perturbation. But the theory predicts that regulation involving changes in the activation gradient be quicker than those which depend on changes in source density. By means of grafting experiments, MacWilliams has been able to compare the time taken by putative high-source regions and putative high-activator regions to regulate down to the same level of activation. In his experiments, the latter took 12 hours for an adjustment that took the former forty. Wolpert's simpler theory cannot account for this result.

Most of those present at the symposium probably accepted that polarity originates in interacting gradients. Brian Goodwin (University of Sussex) offered an alternative based on work with the large single-celled alga *Acetabularia*, in which polarity seems to reside in the membrane sheath. When the cytoplasm of an isolated section of stalk is removed, homo-

Peaks and valleys in morphogenesis

by Miranda Robertson

A symposium on Polarity and Morphogenetic Fields was held at the Hubrecht Laboratory, Utrecht on March 4–6, 1976.

genised and returned, the cap regenerates with the same polarity as before. Moreover the polarity of the cell corresponds to a gradient of phosphate incorporation on the inner side of the membrane, and exposure to a calcium/magnesium ionophore, which reversibly inhibits differentiation, also abolishes the phosphate gradient. Goodwin would like to attribute the phosphate gradient to a propagated wave of action potentials which can be recorded from an external microelectrode. He has suggested a graded "memory trace" in the form of a graded deposit of material left by a propagating wave to explain the establishment of polarity in multicellular animals. But as he acknowledged, there is no evidence for a causal relationship between the wave and the gradient in *Acetabularia*.

To objections that the origin of polarity in a single-celled organism may be irrelevant to multicellular ones, Klaus Sander (University of Freiburg), chairing the session, responded that all embryos develop from a single egg cell. To explain his own results with insect eggs, however, he has joined the majority in postulating gradients rather than propagating waves.

Sander has shown that the antero-posterior polarity of the chironomid midge *Smittia* can be partly reversed by centrifugation, local ultraviolet irradiation, or even a pinprick at the anterior pole of the egg. This can produce embryos with mirror-image duplication of the abdomen instead of a thorax and head.

This pattern was explained by Meinhardt in terms of the Gierer–Meinhardt theory of short-range activation and long-range inhibition. The effect of disrupting the egg is to allow the inhibitor level to drop, creating a second activation peak. The change in slope of the gradient explains the reversal of polarity. But which sequence of segments is specified depends on the gradient level at the point when it is interpreted by the cell. To explain the exact patterns produced by insect eggs disrupted at different times, Meinhardt has had to add to the theory an account of how the cells read the gradient level. He suggests that genes are sequentially inactivated, the number of genes inactivated increasing

with the height of the gradient; but whereas the gradient level can change rapidly on disruption, it is read relatively slowly and because of sequential inactivation, it takes longer to read high values than lower ones. Since in *Smittia*, the gradient is read from posterior to anterior, disruption can thus cause only the specification of more posterior structures.

This particular formulation however works only for size invariant fields. Pattern regulation in insect eggs and *Hydra* is morphallactic: that is, the pattern re-establishes itself by adjustment of existing tissue, without new growth. Willem Ouweneel (University of Utrecht) and Bruce Carlson (University of Michigan) addressed themselves to the rather different problem of epimorphic growth, which occurs in the regeneration of insect and amphibian limbs, and *Drosophila* imaginal disks.

Lively discussion was stimulated by Ouweneel's account of a recent attempt by Vernon French and Susan and Peter Bryant to draw the data from cockroach and newt limbs, and *Drosophila* imaginal disks under the same theoretical umbrella. The theory was developed by French to overcome difficulties in explaining the reliable appearance of supernumerary limbs in the cockroach if one of the transverse axes of the leg is reversed by left-right grafting. Susan Bryant simultaneously and independently arrived at the same explanation for analogous effects in newt limbs. The crux of the theory is that the limb is specified by polar coordinates (the "clock-face" model).

Reversal of polarity of the blastema relative to the stump creates a discrepancy between the positional values of blastema and stump at any given location, and the theory states that that discrepancy is eliminated by intercalary regeneration of a series of values completing the normal sequence by the shortest possible route (Fig. 1). Where the discrepancy causes the regeneration of a complete circle, supernumerary limbs will appear. Polar coordinates

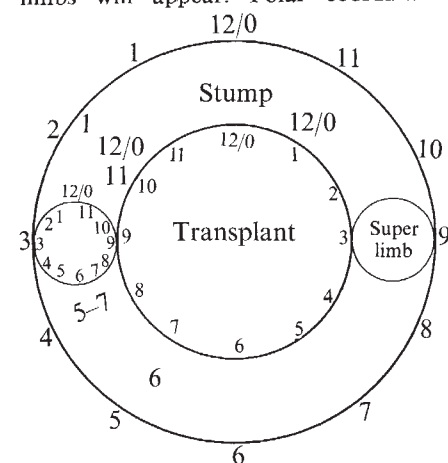


Fig. 1

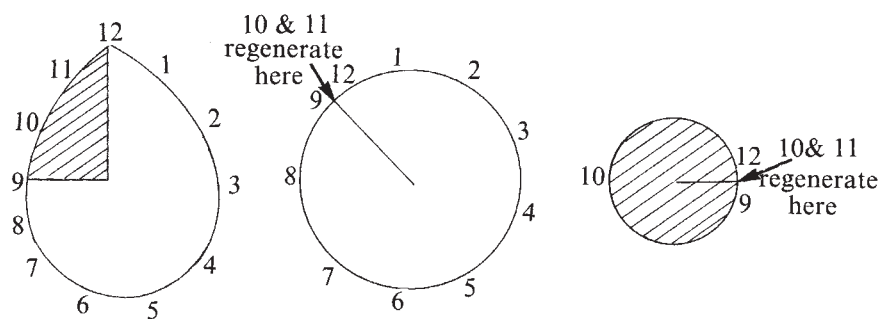


Fig. 2 The wing disk of *Drosophila*. The cut edges of the fragments come together and intercalary regeneration occurs between them.

can also account for the pattern of intercalary regeneration recently reported by Haynie and Bryant for *Drosophila* wing disk (*Nature*, **259**, 659–662; 1976). If a quadrant is removed from the disk and the two fragments allowed to complete their development, the major fragment always regenerates the missing pattern and the minor one always mirror-duplicates the existing one. If the manner in which the cut edges heal is taken into account, intercalary regeneration of polar values accurately predicts this behaviour (Fig. 2). Bruce Carlson's experiments on the regeneration of multiple digits in axolotls give results which however do not entirely conform to the pattern seen in newt blastema. Carlson has explored the effects of rotating separately the different tissues of the stump and finds that the pattern of regeneration is distorted only when mesodermal tissues (muscle or dermis) are rotated. But whereas Bryant finds supernumerary limbs after either antero-posterior or dorso-ventral reversal of the blastema, Carlson obtains multiple digits only from antero-posterior rotation.

S. Strub (Zürich) reported what seems to have been the disruption of the proximo-distal, as opposed to the circumferential, coordinates of the *Drosophila* leg disk by disaggregation of a quadrant of the disk. Such fragments develop entirely distal structures. These are normally specified at the centre of the disk, where French, Bryant and Bryant suggest that the gradient is lowest; but how disaggregation should cause such sinking to a uniform low is not clear.

It was generally agreed that if such puzzles are to be solved by theoretical modelling, the models will have to be formulated in precise quantitative terms, both for the establishment of the morphogenetic gradient and for its interpretation by the cells. Richard Gardner (University of Oxford) pointed out that whereas for example the homoeotic mutants of *Drosophila* could be used to shed some light on the cellular response, mutants affecting

the establishment of gradients are unknown. The best that models can do is to provide logically consistent quantitative descriptions of experimental phenomena, as long as the molecular mechanisms of morphogenesis remain inaccessible. It is reported that a British Forces team will shortly attempt Everest without oxygen. □

Inverse square law for gravity?

from a Correspondent

'EVERY schoolboy knows' that gravitational attraction varies inversely as the square of the distance between the attracting masses—or at least it used to be common knowledge. Newton himself first gave strong support to the 'inverse square law' by comparing the rate at which the Moon accelerates towards the Earth with gravitational acceleration at the Earth's surface. He later provided compelling evidence by showing that Kepler's laws for the motion of the planets followed from the same law of attraction—that is, that they would move on ellipses with the Sun at one focus, and the square of their periods would be proportional to the cube of their major axes. Subsequent study of the motion of the planets and satellites has confirmed the truth of the inverse square law at astronomical distances (say from 1,000 km upwards) to a high degree of precision. It is therefore intriguing that in an article by D. R. Long in this issue of *Nature* (page 417), the question of the validity of the inverse square law at laboratory distances has been raised. An examination of the most accurate laboratory determinations of the constant of gravitation, G , suggests a systematic shift with the separation of the attracting masses—the larger the

separation, the larger the value of G . In other words, Long suggests that the inverse square law is not valid, and that we have to add an additional short-range repulsion. The experiment reported here confirms his suggestion, by comparisons at distances of 4.5 and 30 cm.

The question of a deviation from the inverse square law has been raised most seriously in 'recent' times by Newcomb, who showed that a small change in the form of the law could explain anomalies in the motion of Mercury. These anomalies are explained naturally by Einstein's General Theory of Relativity, which may be interpreted in a sense as predicting deviations from the inverse square law of gravitation. However, at laboratory distances with laboratory masses, Einstein's theory predicts that the inverse square law should hold to a high precision. Long's suggestion will therefore not meet with ready acceptance. If substantiated, the implications, for example, for our knowledge of the interiors of the Earth and the Sun would be considerable. We infer their masses from our knowledge of the constant of gravitation, and Long's experiment indicates that the limiting values of G at large distances may be several percent higher than is presently accepted. Moreover the implications for a quantum theory of gravity would be serious.

One is tempted to echo the reaction of the bishop's wife to the suggestion that man is derived from the apes—'let us hope that it is not true—or if it is, that it won't become generally known.' More responsibly, we should affirm our willingness to submit theory to experimental proof, and hope that Long's suggestion will stimulate a critical experimental appraisal. He has drawn attention to the remarkable fact that there is no accurate experimental evidence for the inverse square law of gravitation at laboratory distances. By comparison, very accurate null experiments on the basis first indicated by Cavendish have shown that the exponent in the inverse square law for electrostatics differs from two by less than 3×10^{-16} (Williams, Faller, and Hill, *Phys. Rev. Lett.*, **26**, 721; 1971). Can anyone devise an accurate null experiment which will confirm (or deny) the inverse square law for gravitation? □

Erratum

In the article 'Disappearing habitats' (*Nature*, **259**, 365; 1975) the floral survey mentioned was carried out by Mr and Mrs C. Scotter and not by P. M. Wade.

Mason develops Milankovich Ice Age theory

from John Gribbin

ON March 17 Professor B. J. Mason, Director-General of the UK Meteorological Office, delivered this year's Symons Memorial Lecture, in which he presented strong evidence in favour of the 'astronomical' theory of Ice Ages, with his own improvements on the Milankovich model.

Mason's talk, titled "Towards the understanding and prediction of climatic change" covered some familiar ground both at the beginning, where he restated his position in the debate about whether the sunspot cycle and

other solar fluctuations affect climate (see *Nature*, 259, 367; 1976) and at the end, where he stressed the value of computer modelling in our developing understanding of the atmosphere. Professor Mason believes that this is the only way in which we may be able to understand how the climate works although there is a long way to go yet before unique predictions will be possible. In some ways, the centre-piece of the talk was also familiar, at least to readers of *Nature*, in that it dealt with the Milankovich theory of Ice Ages. But Mason presented new, unpublished evidence in support of the theory, and also offered a uniquely persuasive insight into how the energy fluctuations involved could drive both the onset and decline of Ice Ages; it may well be that as a result, March 17, 1976 is remembered as the day when Milankovich's model, after four decades of controversy, at last became established as the leading contender to explain climatic fluctuations with timescales from a few tens to a few hundreds of thousands of years.

The key to this development has been the availability in the past few years, for the first time, of reliable continuous records of both temperature and total ice volume fluctuations covering the past 10^5 yr or more. These are the records obtained by interpreting isotope fluctuations (notably the $^{18}\text{O}/^{16}\text{O}$ ratio) in both ice cores and in marine cores containing the remains of small creatures such as plankton. The three separate variations in Northern Hemisphere insolation produced by changes in the orbit and inclination of the Earth have periods (in round terms) of 96,000 yr, 40,000 yr and 20,000 yr; all three of these periods (and no others as significant) were present in a power spectrum analysis of the planktonic data which Mason had obtained from N. Shackleton in Cambridge (as yet unpublished). Mason stressed the importance of this link between statistically significant periodic variations and a known physical mechanism, contrasting the situation with that of some of the more short term data where seemingly significant peaks often have no physical explanation, and peaks expected on physical grounds (such as the 11-yr solar cycle influence) often are not significant. With this incentive, Mason has reworked some aspects of the Milankovich model in terms of variations in the amount of heat received at different Northern Hemisphere latitudes in different seasons, using both the original astronomical data of Milankovich and modern improvements on the data. North of 45°N the variations due to these effects cover about 4×10^{18} calorie d^{-1} (1.5×10^{21} calorie yr^{-1}), which is about 1% of the total heat received by the

polar cap, and Mason reported that he was "staggered" to find the very exact agreement over the past 150,000 yr between times of minimum insolation and the maximum advance of Northern Hemisphere ice.

Mason says "this is impressive, and the last thing I expected" and that the remarkable agreement encouraged him to look at the integrated effect of insolation variations during periods in which the ice cover advanced and retreated. Again for the region north of 45°N , when ice cover developed during the period from 83,000 BP to 18,000 BP the integrated deficiency in insolation produced by Milankovich variations amounted to some 1,000 calorie for each gram of ice formed using Milankovich's figures, or some 556 calorie g^{-1} with the modern figures. Since the latent heat of the water/ice transition is 677 calorie g^{-1} , the figures are impressive even without allowing for the possibly crucial significance of the fact that the variations in insolation are much greater in summer than in winter.

From 18,000 to 6,000 BP, when the ice was melting, the integrated 'excess' heat received was 4.2×10^{24} calorie on Milankovich's figures and 10×10^{24} with the modern astronomical figures, while the latent heat required for the known decrease in volume of the ice was 3.2×10^{24} ; a coincidence which stretches across 24 orders of magnitude to agree so closely is certainly not to be dismissed lightly, and work is now in progress at the Meteorological Office to produce a more detailed model taking account of the capacity of the ocean to act as a reservoir of heat and the feedback mechanisms between ice and ocean. Mason even ventured if not a prediction then at least a comment on future climatic fluctuations, pointing out that we are now shifting from a situation in which the Milankovich effect has produced excess insolation in the North (above the mean for the past 150,000 yr or so) to one in which there is a deficit. Simple astronomical calculations show the dip reaching a Northern Hemisphere insolation minimum in about 10,000 yr, but bottoming out before reaching conditions quite so extreme as those which coincided with the maximum advance of the most recent Ice Age. Many members of the packed audience for Mason's lecture will undoubtedly be eagerly awaiting the full model now being developed by A. Gilchrist and others at the Met. Office; some, no doubt, will now be taking a fresh look at the Milankovich model themselves. Meanwhile, in Mason's words, "this effect cannot be laughed away... certainly all the energy quantities are in the right ballpark". □



A hundred years ago

LORD SALISBURY, on Monday, named the following as Commissioners under the Oxford University Bill:—Lord Selborne (Chairman), Lord Redesdale, the Dean of Chichester, Mr. Mountague Bernard, Sir Henry Maine, Mr. Matthew White Ridley, and Mr. Justice Grove. The feeling among scientific men is one of intense disappointment, leading to the conclusion that it is useless any longer to consider whether Oxford will ever be in a position to do anything for the promotion of science.

THE report of the Cambridge Board of Mathematical Studies to the Studies' Syndicate contrasts with the reports of most of the other boards in the paucity of its suggestions for improvement. They do not seem to think that very much is required in order to perfect the system of mathematical teaching. They believe in the probable stability and development of the system of inter-collegiate lectures, but say very little to assist its development, and they say nothing about the present vehement competition by means of private tuition, and the defective method of study that it induces. In answer to the question how university teaching may be organised so as to give the greatest encouragement to the advancement of knowledge, "the Board offer no suggestions under this head." Is this quite what might have been expected in a report bearing the signatures of Stokes, Cayley, Adams, Clerk-Maxwell, Sir W. Thomson, Tait, Lord Rayleigh and James Stuart? May there not be some obvious explanation of this phenomenon? The whole report consists of only forty-one lines.

from *Nature*, 13, March 30, 434-435; 1876

articles

Visual illusions in geology

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Examples of visual illusions in the laboratory are well documented, but what about illusions that occur during field observation of geological structures? In this article I discuss a study involving geologists and non-geologists and define some of the problems that may arise during geological field and laboratory work.

THOUGH visual illusions have been studied mostly in controlled laboratory situations^{1,2}, Poggendorff, Zöllner and other illusionary distortions can also occur during observations of rock formations. Depending on the interaction of psychological and geological hypotheses, these can lead to false deductions. Problems may arise especially in the observation of elliptical ooids, crenulation cleavages and intersecting veins and fractures. Illusions in geological contexts are of psychological interest: judgements of area, for example, are found to be the reverse of those predicted on the basis of the Helmholtz irradiation illusion.

Four versions of the Poggendorff illusion in geological contexts are shown in Fig. 1. The inclined traces in Fig. 1b seem to be offset across the stippled bands. Were the bands representative of veins then these illusory offsets could lead to an interpretation suggesting dilational origin, whereas replacement is more appropriate. To some observers the linear elements seem also to be slightly rotated anticlockwise in the centre gap. This could be taken to imply the operation of shear strain of a different magnitude within and outside of the stippled layers. In Fig. 1c the rock cleavage traces seem to be displaced by interfacial shear at the layer boundaries, especially at the arrowed locations. The illusory displacements in Fig. 1d occur across the dark layers, suggesting a greater independence of the strain histories of the lighter layers above and below, than is justified; in fact, the cleavage traces are precisely in line.

The context effect of the fault at A in Fig. 1a and the gap in data caused by, say, a river course, increases the probability of an incorrect fault inference at B. The stippled layer is straight and continuous. For purposes of terminology in psychological studies it is convenient, however, to take the data gap band in the Poggendorff illusion as analogous to a geological fault. Then the displacement directions in this illusion can be described by labels which are usually reserved for tear faults. The line on the far side of the data gap is sinistrally displaced ('sinistral Poggendorff') when the apparent offset is to the left, dextral when it is to the right. Although such illusory offsets and rotations can be minimised by viewing along the interrupted linear elements—with the direction of view positioned at a low angle to the rock surface—this is rarely done when observing cleavages, since refraction is expected.

When exploratory mineral and petroleum investigations are at the reconnaissance stage, the use of image analysing computers for percentage area estimation is uneconomical. Visual estimations of rock porosity and percentage volume occupied by a diagnostic mineral are frequent, and monochromatic^{3,4}

and chromatic percentage area comparison charts have been devised to facilitate judgment. But monochromatic charts invariably present black grains on a white background, and single black shapes on white appear smaller than equal area white shapes on black (Fig. 2).

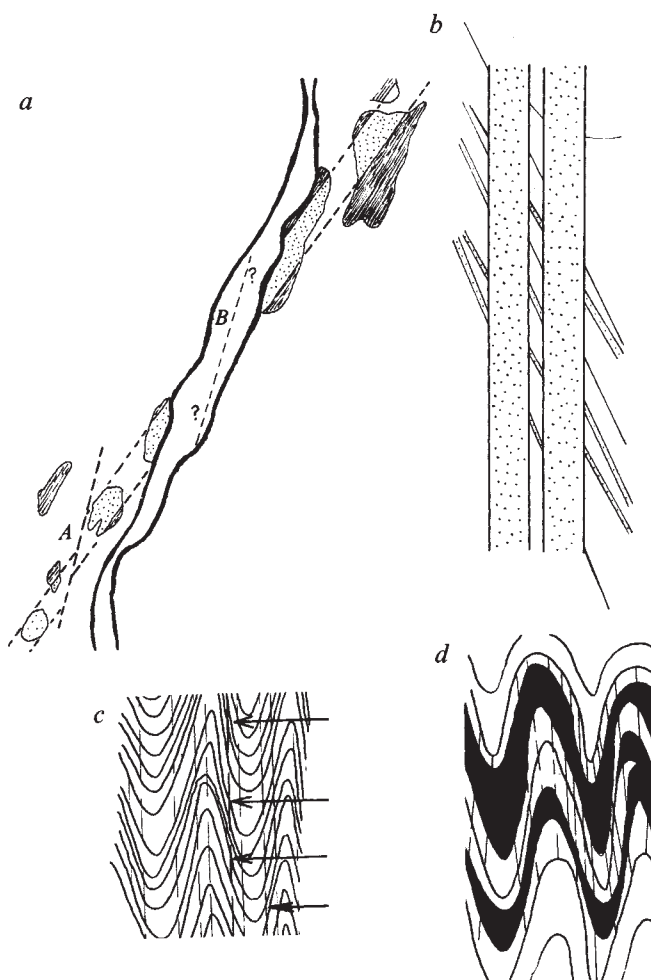


Fig. 1 The Poggendorff illusion in geological contexts; the illusory perceptual data are more likely to mislead when they favour an investigator's geological hypothesis than when they do not. *a*, 'Sinistral Poggendorff' offset of stippled layer across data gap misinterpreted as a fault, an interpretation reasonable at A, but not at B; *b*, illusory displacements, continuations and rotations of linear elements across two adjacent data gaps suggest dilation effects; *c*, illusory step-like displacements of cleavage, especially at locations arrowed; *d*, apparent offsets of continuous cleavage traces across dark bands.

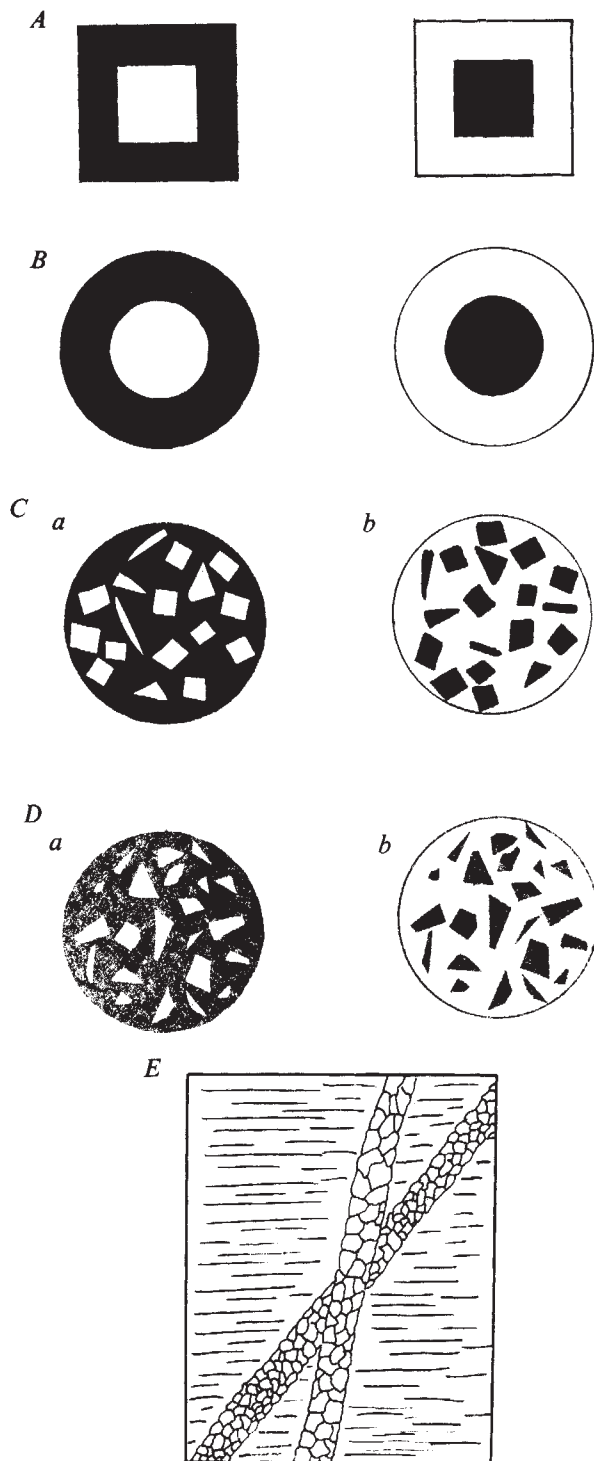


Fig. 2 *A* and *B*, The Helmholtz irradiation illusion in which a light area on a darker background appears larger than an equal dark area on white; however, in *C*, estimates of the percentage area occupied by grains in *a* and *b* are usually equal when both are presented together; but when presented separately the black grains (on the line-bounded background) are judged to cover a larger area than the white grains (on a contrast-bounded background); *D*, the grains in *a* and *b* again, in spite of the lower brightness contrast than in *C*, seem to cover the same area, but the dark grains are judged to cover a greater area when judgments are made separately. Actual area in *c*: 25%; *d*, 20.5%. *E*, 'Sinistral' Poggendorff offset of the older vein in *E* was misremembered as dextral by 23% of geologists.

Although this may be expected to cause an overestimation bias in area estimations of lighter grains, or areas, within a darker matrix, this is not necessarily the case. Twenty-eight

geologists (with 2–5 yr experience) individually gave unaided absolute estimates of the total areas occupied by grains in eight 5-cm diameter items (9–23 grains in each). Two of the samples (17 and 23 grains) were presented deliberately both in white on black and black on white, with the grains evenly distributed. Contrary to predictions from the effect in the Helmholtz irradiation illusion, mean area estimates were larger (and not smaller) for black grains on a white background than for white on black. Overall mean values were respectively 29.4% and 25.4% ($0.025 > P > 0.01$, actual area 25%). Over this range of grain density the act of estimation may therefore involve other perceptual and cognitive processes which cause closure between multiple black elements and which thus counter the mechanisms used in seeing the standard irradiation illusion.

Monochromatic charts with black-on-white figure-background relationships should be misleading when total area estimations need to be made of a few large items; they do not

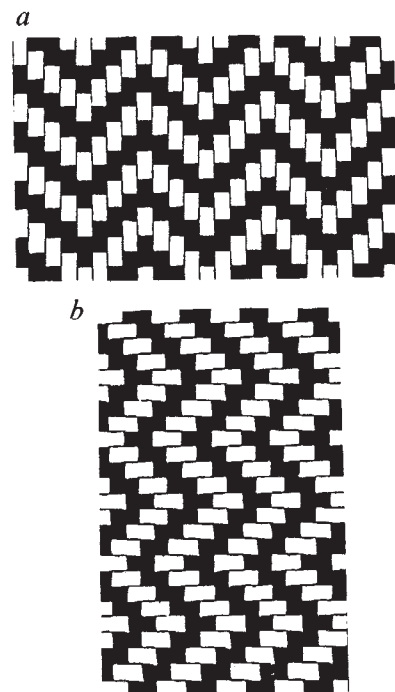


Fig. 3 *a*, The "illusion of the kindergarten patterns" is schematically similar to fracture cleavage associated with large offsets. The apparent fanning of the laths is entirely illusory; all are parallel sided and aligned vertically. Decreases in brightness contrast between, say, lighter sandy layers and darker clay-rich layers, decreases the illusion. *b*, The same as *a* but at 90° to it.

accord so closely with estimates made without their aid as do the white-on-black scenes. The latter, in fact, are less likely to cause an underestimation bias for scenes of intermediate magnitude. Imagery-based techniques, such as mentally translating grains so that they cluster and fuse into a sector, do not promote greater accuracy of performance. Subjects stated that mental translation led to increased errors when the grains were small, widely separated, circular, or of uneven outline. Estimation through general impression, using 3–4-s delayed augmented feedback during 15 estimations of chromatic and monochromatic natural and diagrammatic samples, led to lower errors in other subjects after unaided estimates had stabilised (before-after comparison for 15 geologists (>3 yr experience) and 2 non-geologists (one male, one female), ($0.025 > P > 0.01$)). The two female subjects tested had the highest initial errors but improved more than all the male subjects, eventually equalling male performance.

If illusory data elements contradict common geological expectations, then the latter dominate over some of the psychological

elements, during constructions in visual memory, but not necessarily over others. A total of 69 geologists with 2–5 yr experience observed a slide depicting two acutely intersecting but straight, undisplaced veins of equal thickness (Fig. 2e). The intended Poggendorff offset is sinistral. After viewing for 7 s they drew representations which were assessed by three neutral judges for unambiguity of age relationships and true continuity of the older (more gently inclined) vein across the younger (near vertical). Twenty subjects did unambiguously represent the older vein as displaced but 16 of them showed the displacement as dextral. Such a representation should be expected only if the younger vein grows at a high angle to its margin. Six geologically and psychologically inexperienced subjects reported seeing the “sinistral displacement” but four of these could not accurately remember data on age relationships (the latter was accurately recorded by 96% of the geologists).

Figure 3 shows the combined effect of the irradiation distortion and Poggendorff offset in the form of the “illusion of the kindergarten patterns”⁶. This is schematically similar to those instances of fracture cleavage in deformed rocks where the displacement along the cleavage is large in comparison to the thickness of the cleaved layer, and the brightness contrast of the rock lithologies is high. The ‘fanning’ of the cleavage is illusory: all the cleavage surfaces are straight, parallel and vertically in line. Similar problems may be created by geological analogues of the Zöllner and Fraser illusions (ref. 2 and Fig. 4). The tightly folded recumbent multilayer in Fig. 4a, for instance, seems to be penetrated by a convergent cleavage fan; that in Fig. 4b seems to be penetrated by divergent zones of thinning. In fact, the cleavage traces are parallel and in line (that is, ‘axial planar’). Structures with these general appearances are quite common in orogenic belts, yet true axial planar cleavage is rarely reported. Its frequency of occurrence may have been underestimated. This emphasises the importance of the precise measurement of cleavage fan angles, in contrast to reliance on visual judgment and general verbal labels.

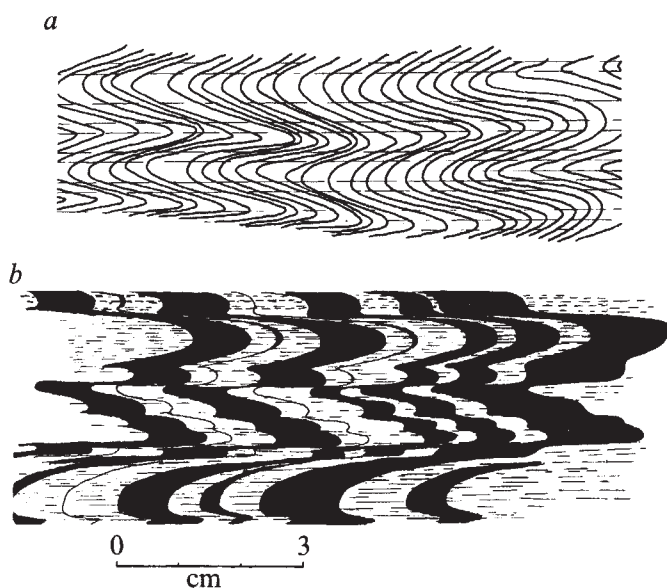


Fig. 4 Geological analogues of (a) the Zöllner illusion, and (b) the Fraser illusion. *a*, Recumbent multilayer folds seems to have an associated convergent cleavage. In fact, all the cleavage traces are parallel (true axial planar cleavage). Easily overestimated convergence angles are also seen in many outcrops of mesoscopic F_2 folds in the area of the Ballachulish “nappe”, Argyllshire, Scotland. *b*, Based on a natural structure in Appin Quartzite Transition Group, Laroach Valley, Argyllshire. Six out of seven postgraduate structural geologists perceived the four major crenulation cleavage zones as fanning, and the finer, small scale cleavage traces as parallel sided. In fact, finer traces are fanning (divergently) and lower order zones are parallel sided.

In the vertical–horizontal illusion a vertical or slightly left-of-vertical line⁶ appears longer than a horizontal line of the same length. Consequently, the amplitude–wavelength ratio of sandstone ripples, or indeed any waveform, seems to be larger when the enveloping surface to the waves is horizontal, than when it is vertical. Low eccentricity ellipses, with horizontal long axes also appear closely circular when the difference between axes lengths is small ($< 5\%$). Geological objects known as ‘ooids’ (Fig. 5a), which have circular outlines when undeformed, become elliptical after compaction and tectonic deformation. They are, however, rarely geometrically ideal shapes, and in some cross sections their interiors may be very irregular or even more elliptical than the peripheries. In the latter case this results in an illusory contraction of the ooid normal to the long axis of the interior ellipse—a variant of the Delboeuf illusion⁷.

In most real geological situations, however, specific and nonspecific context effects do influence perceptual judgments. An important nonspecific effect is the continual necessity for separating out non-random fluctuations in shape and orientation from the inevitable random visual ‘noise’ inherent in geological (as opposed to geometrically ideal) shapes. Signal non-randomness in ooid geometry can go undetected when axes lengths differ by between 5% and 15% if geologists, through training, become accustomed to perceiving non-ideal forms and thus too easily accept natural shape irregularities as noise. Out of 54 professional and postgraduate structural geologists asked individually if the ooids in Fig. 5a needed analysing for evidence of deviations from circularity and of non-randomness in orientation, 36 replied that they did not (Fig. 5c). In fact, all the ooids are elliptical (Fig. 5b) and there is a preferred orientation of long axes in the horizontal. This fabric would not be eliminated no matter how high any subsequent strain and so their analysis is necessary. Sixteen non-geologists asked the same question all replied that they did think the ooids required analysis (confidence levels ≥ 3). This would be explicable if the non-geologists were comparing the stimuli with more ideally shaped, memorised prototypes.

The practical way to aid decisions in these circumstances is for the observer to experiment with himself by judging the axial ratios of those ooids which to him seem closest to a unit axial ratio (however irregular their peripheries) and then checking the judgment by measurement. Errors of up to 15° commonly also occur in the location of the long axes of ooids when axial ratios are below 1.3:1. If two possible ‘long axes’ are constructed at the limits of the zone of location uncertainty, the length of the bisector of these axes is a better measure than that of one located intuitively.

On the basis of inappropriate constancy scaling interpretations of illusions¹, the upper half (‘further away’) portion of a vertical line should appear longer than the lower half. In addition, subjects can be expected to locate mid-points too high up along a vertically oriented dimension. Some evidence for the presence of such an effect, in a geological context, arose during an investigation⁸ in which professional geologists located inflection points too high up on the limbs of moderate and high limb dip folds. Bisections were made within zones ranging from < 0.5 cm to 3 cm in length. In order to test both for range effects and also for possible psychological hindrances in the use of an additional way-up determination technique (Fig. 6), 30 final-year undergraduate geologists were requested to locate a number of mid-points between parallel lines separated by 3 cm and 6 cm. They were to judge the mid-height point. The parallel lines could be horizontal, inclined (45°) to the left, inclined to the right or vertical. Each of the 30 subjects provided 2–10 observations for each of the 8 conditions. No bias was found for the oblique or vertical cases, but in both width conditions, there was a small but very consistent tendency to locate mid-points too high up between horizontal lines (mean error 1.6%, $0.01 > P > 0.001$; all other conditions showed mean non-constant location errors of $< 1\%$).

In an experiment examining the detection of asymmetry

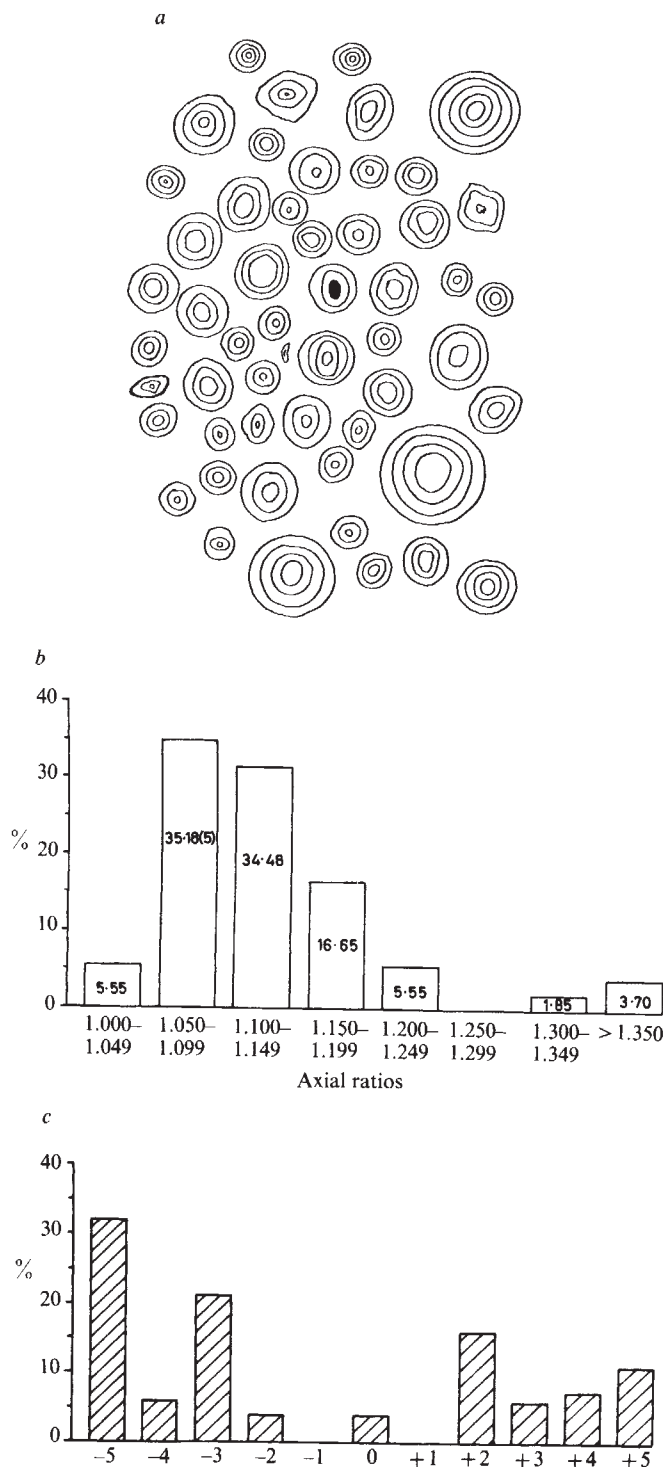


Fig. 5 *a*, In reflection of the vertical-horizontal and Delboeuf illusions, slightly elliptical ooids appear more closely circular than really they are. *b*, Frequency histogram of ooids' axial ratios, none of the ooids in *a*, are circular (54 ooids, mean axial ratio 1.15, range 1.025-3.45). *c*, Decisions of 54 geologists as to whether or not to analyse the ooids in *a* for evidence of inherited non-random deviation from a circular outline (-5 to +5, increasing levels in confidence of decision: -5, "definitely would not analyse"; +5, "definitely would analyse").

in boudinaged layers (Fig. 6b), similar results were obtained when 10 non-geologists and 3 postgraduate geologists assessed whether the zones of deflection around boudinaged layers (the waves dying out on both sides of the broken layer in Fig. 6b) had the same or different distances of penetration (stimulus visual angle 12°). Each subject provided two observations for

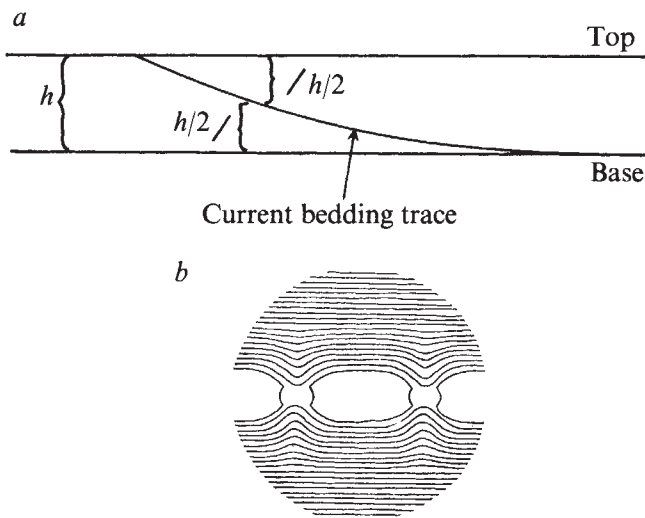
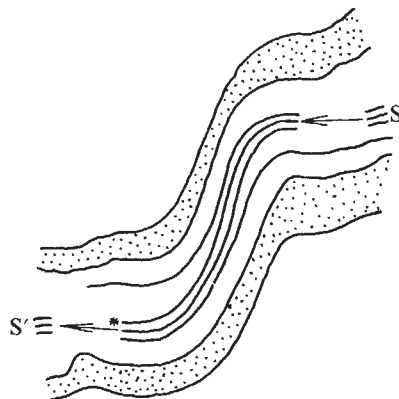


Fig. 6 *a*, 'Mid-point' method of way-up determination in areas where younging evidence is sparse and current bedding strongly flattened, demonstrated on an unambiguous example. Where mid-height surface intercepts current bedding trace, the longer segment is in the older half of the bed. Locations of mid-points by eye in horizontally positioned units tend to be too high up, leading to incorrect inferences of younging direction. *b*, One of the boudinaged layer stimuli: if deflections have less penetration above a boudinaged layer than beneath, this asymmetry is less likely to be detected than if the converse is true.

each of four width conditions (same, 10, 20 and 50% difference) and four orientation conditions (horizontal, left and right oblique, vertical) in tachistoscopic presentation. The mean accurate decision time for the horizontal cases was 4.8 s, but for both oblique and vertical cases was 4.6 s. When, as in Fig. 6b, the upper zone of the horizontal condition was 10% narrower than the lower, 31% of the responses were erroneous; when the two were equal, 27% were erroneous. But when the lower half was the narrower, errors were reduced to 11.5%. This also indicates that when waves with progressive amplitude changes, cross the vertical dimensions to be compared, misjudgments of the length differences become exaggerated. The processes causing this may include differences in the implicit use of cues to upward as compared with downward perspective changes.

Provided that these perceptual difficulties are considered, it is possible for the geologist to use the mid-point location as an aid to way-up determinations. When current beds are strongly flattened it becomes more difficult to differentiate between their asymptotic bases and truncated tops. The way-up can, however, be determined by locating the mid-point between

Fig. 7 During lengthy visual tracking of layers in air-photographs and outcrops eye movements occasionally "jump tracks" and continue along adjacent layers. During visual tracing of the layer *S*, starting at top right, eye movements may continue at bottom left along *S'*. Correct line is asterisked.



the flanking bedding traces. Where this mid-height surface intersects the current bedding trace it divides it into two unequal segments, and the longer segment is in the older half of the bed (Fig. 6). I have validated this technique on flattened current beds in the Moine and Dalradian quartzites of the Loch Dochart region of Argyllshire, Scotland. If, however, the flanking bedding traces are horizontal then the mid-point between them should be located by measurement.

If illusions are conceived in terms of inappropriate visual induction processes, then they are difficult to compensate for because the false hypotheses on which they are perceived are in other circumstances correct, useful, and also out of conscious control¹. Hypotheses, some of which can be brought into awareness, are also generated during visual search⁹ and visual pursuit. In Fig. 7 a common assumption is that the three-layer batch at top right continues at bottom left. On the basis of this incorrect hypothesis the observer's eye movements shift from *S* to *S'*. When a wave of this geometry was present in a longer pursuit task, it caused shifts in eye movements, to adjacent layers, in 9 out of 29 undergraduate geologists. Visual pursuit needs to be done accurately during the interpretation of air-photographs and also during the observation of complex refolding relationships in outcrop. In the latter circumstances,

pursuit lapses can lead, for instance, to 'mushroom' interference patterns¹⁰ (contorted, but closed outcrop patterns) being missed when present, or spuriously inferred when absent. Because of difficulties in the visual pursuit of schistosity through the zone of thinning, crenulation cleavage often seems to involve actual limb displacements in items where no such displacements exist. Observers' eye movements (saccades) shift from their intended course, especially when the track is hypothesised to be more predictable than really it is. To avoid these shifts it is useful either to look along the track at a low viewing angle or deliberately to concentrate on the minutiae of any irregularities. The latter technique reduces saccade distance through eliminating the hypothesis that the track is highly predictable.

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- ¹ Gregory, R. L., *The Intelligent Eye* (Weidenfeld and Nicolson, London, 1970).
- ² Robinson, J. O., *The Psychology of Visual Illusion* (Hutchinson, London, 1972).
- ³ Folk, R. L., *J. sedim. Petrol.*, **21**, 32–33 (1951).
- ⁴ Terry, R. D., and Chilingar, G. V., *J. sedim. Petrol.*, **25**, 229–234 (1955).
- ⁵ Pierce, H., *Psychol. Rev.*, **5**, 233–253 (1898).
- ⁶ Pollock, W. T., and Chapanis, A., *Q. Jl expl Psychol.*, **4**, 170–178 (1952).
- ⁷ Sanford, E. C., *Sensation and Perception* (Boston, Heath, 1903).
- ⁸ Chadwick, P. K., *Nature*, **256**, 570–573 (1975).
- ⁹ Noton, D., and Stark, L., *Science*, **171**, 308–311 (1971).
- ¹⁰ Ramsay, J. G., *J. Geol.*, **60**, 466–481 (1962).

Man-made e.l.f./v.l.f. emissions and the radiation belts

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Morphological studies of e.l.f./v.l.f. emissions on the Ariel III and IV satellites indicate that man-made electromagnetic emissions, namely power-line harmonics generated in the industrialised regions of North America and, also, v.l.f. transmissions at 17.8 kHz (NAA) and 16.0 kHz (GBR) in the longitude sector which encompasses the South Atlantic Anomaly, are responsible, at least in part, for the formation of the electron slot ($2 < L < 3$) between the inner and outer radiation belts in the magnetosphere.

A PRELIMINARY study by Lefeuve and Bullough¹ of v.l.f. emissions at 3.2 ± 0.5 kHz and 9.6 ± 0.5 kHz recorded on the Ariel III satellite (altitude ~ 500 km, orbital inclination 80°) revealed the existence of a permanent zone of v.l.f. emission occurring over the USA and its geomagnetically conjugate region in the Southern Hemisphere. These Ariel III studies have since been extended and now confirm the importance of this longitude sector for wave-particle phenomena at low *L* shells ($2 < L < 3$). This American zone of e.l.f./v.l.f. emission is shown most clearly on the maps of percentage occurrence (Figs 1 and 2) for quiet magnetic periods ($K_p \leq 2+$). To obtain Figs 1 and 2, a count was made of the number of observations (typically 25–40) in each $10^\circ \times 2^\circ$ longitude–latitude sector and the percentage of such occasions for which the signal intensity exceeded $10^{-11} \gamma^2 \text{ Hz}^{-1}$ FSE found. (FSE: free space equivalent, thus $10^{-11} \gamma^2 \text{ Hz}^{-1} \text{ FSE} = 4.8 \times 10^{-15} \text{ W m}^{-2} \text{ Hz}^{-1}$ for a circularly polarised wave.) The orbit of Ariel III was such that full local time coverage was obtained in each 80-d period analysed. Little importance can be attached to the very localised

maximum at 5°E , 56°N which occurred in the quiet period following a magnetic storm and close to a ground station at Winkfield, UK where observations were limited by tape recorder read out.

In Fig. 1 the 20% contour of occurrence is centred rather accurately on the North American continent (shown dashed) with a corresponding conjugate region in the south. The 60% contour in the Northern Hemisphere mapped geomagnetically into the opposite hemisphere shows isomorphism in the two conjugate regions. There is a pronounced maximum in occurrence ($> 60\%$) on lower *L*-shells ($2 < L < 3$). A closer examination of the contours of occurrence over North America (Fig. 2) shows that it is possible to attribute the source of the emissions to a combination of power-line harmonics and thunderstorm noise. The two major centres of thunderstorm occurrence (Colorado Mountains and the south-eastern USA) are four times more active in the first period (1967 May, June, July) than in the second (August, September, October). In the latter period the 60% contour is situated above the most highly industrialised region of the USA and south-eastern Canada. In the earlier period its extension to the west and south could be attributed to the greater thunderstorm activity. Electrical power consumption in Canada is concentrated in the south of Ontario and Quebec. Available statistics do not enable us to display it on the map. Measurements by D. Morgan (private communication) in a comparable industrialised area in the UK yielded magnetic field strengths at the ground of about $10^{-11} \gamma^2 \text{ Hz}^{-1}$ for the 3.2 ± 0.5 kHz channel. This is the same as the baseline we have used for Figs 1 and 2. For typical high thunderstorm activity the mean thunderstorm noise level, measured overhead on the Ariel IV satellite without wave amplification, is also $\sim 10^{-11} \gamma^2 \text{ Hz}^{-1}$ FSE. A preliminary examination of 500–1,000

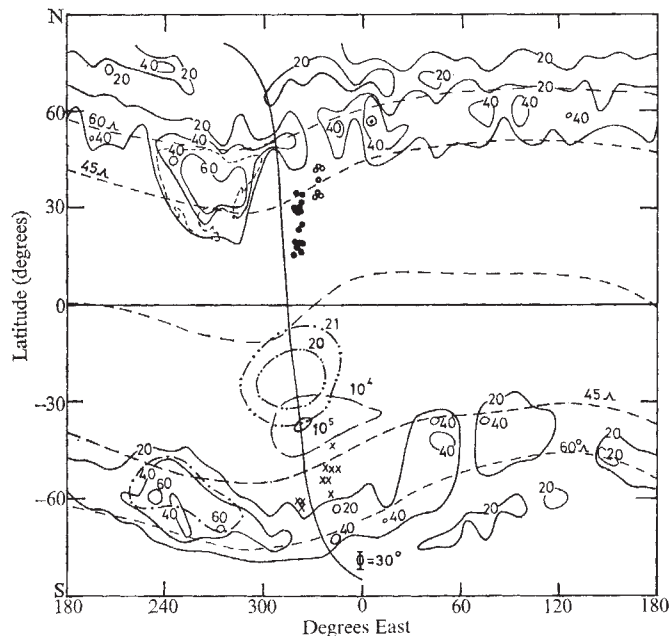


Fig. 1 Percentage occurrence of emissions with intensity $> 4.8 \times 10^{-15} \text{ W m}^{-2} \text{ Hz}^{-1}$ at 3.2 kHz; 1967 May 5–July 24, $K_p \leq 2+$. — — —, 60% contour (Northern Hemisphere) mapped into Southern Hemisphere (magnetic conjugate). - - - - -, Brazilian magnetic anomaly [magnetic field at 600 km ($\times 10^{-6} \text{ T}$)]. - · - · - ·, South Atlantic anomaly [omni-directional electron flux $> 0.5 \text{ MeV cm}^{-2} \text{ s}^{-1}$ at 200 km (Vette, 1967)]. ●, 1967 May 16–23; ○, 1967 June 7–8 inclusive [GBR (code)]; ×, 1971 December 12–1972 March 1 [NAA (FSK)].

Hz e.l.f. data from Ariel IV (1972 March 20–June 22) indicates a small maximum in occurrence for signals $> 10^{-11} \gamma^2 \text{ Hz}^{-1}$ FSE (but none for signals greater than $10^{-9} \gamma^2 \text{ Hz}^{-1}$ FSE) of 85–90% compared with an average of 65% around the world (at $L = 3$). Amplification at e.l.f. is much less than at v.l.f. (see below) and the signal could correspond to unamplified power-line harmonics. Thus the amplified emissions may be band limited in agreement with the rather limited observations from OV1-14 (ref. 2).

Dependence on geomagnetic activity

A detailed analysis of all passes over the zone maximum (240–300°E, 30–45°N) for both quiet and disturbed periods revealed a wide range in signal intensity ($\sim 50 \text{ dB}$) which reflects the variation in magnetic disturbance ($K_p = 0+$ to 9+) in this period. All measured intensities lay above the receiver threshold and a 10–20-dB reduction in the weakest signals from night to day could be ascribed to D-region absorption of a signal transmitted from the ground; this signal level being that obtained in the absence of amplification during quiet periods. Because of this wide range in signal intensity it was not possible to identify either a diurnal or weekly variation in signal intensity which could be attributed to the expected variations ($\leq 3 \text{ dB}$) in power consumption. It is clear that wave amplification (two-hop whistler signal for a source in the USA) occurs very frequently. We have not yet analysed the one-hop whistler signal for this period. An analysis similar to that of Fig. 1 but for disturbed periods ($K_p \geq 3$), shows the percentage occurrence rising to 100% over the eastern USA and its conjugate region. During these disturbed periods there is a tendency for the evening activity to lag behind that of the morning—as for higher latitude events³.

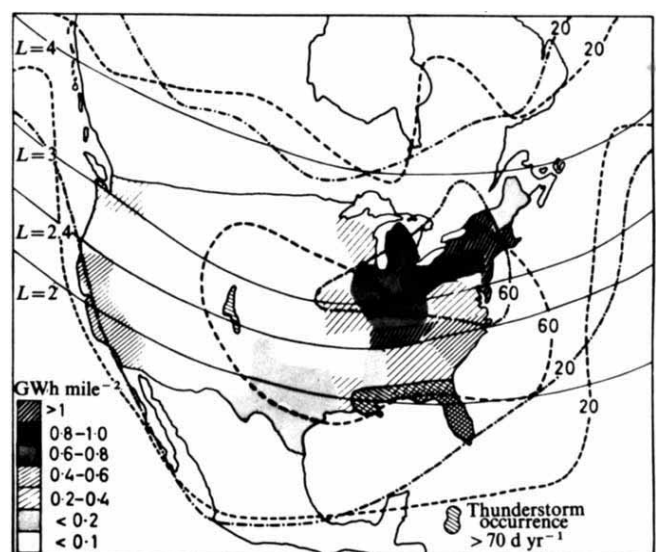
GBR (16 kHz) and NAA (17.8 kHz) wave amplification

Further evidence of the importance of man-made v.l.f. emissions in the electron slot has been found in a study of the GBR (Rugby, 16 kHz) and NAA (Maine, 17.8 kHz) wavefields above

the ionosphere. Amplification of the NAA signal at 17.8 kHz is commonplace whereas that of the GBR signal is rare. The phenomenon is strongly localised in the invariant longitude sector which includes the South Atlantic magnetic anomaly. This is shown very clearly in Fig. 3 where Ariel III daytime (SZA $< 90^\circ$) GBR signal intensities measured in the azimuthal sector 225–255°E of N in the period 1967 May 12–August 1 are compared with those observed in the sector 105–135°E of N. Two types of signal, frequency shift keying (FSK) and specially coded, are plotted. The coded signal consists of pure continuous wave transmitted in a 90-s ON, 30-s OFF sequence. It can be seen that only the coded signals in the azimuth–range sector extending over the North Atlantic exhibit amplification. The observations were made using a minimum reading circuit⁴, therefore transient amplification events in each observing period of 28 s are excluded. The FSK signal intensities lie close to those expected for propagation in the earth–ionosphere waveguide with partial transmission through the ionosphere. The most strongly amplified ($> 10 \text{ dB}$) signals are plotted on the map (N. Atlantic) in Fig. 1. There are two groups; that to the immediate east of the anomaly are events observed at dawn in the period May 16–23 inclusive when the local time and solar zenith angle were ~ 0600 and $\sim 85^\circ$ respectively with little magnetic activity ($K_p \sim 2-$, $\leq 3+$). Further east is a group occurring on June 7 and 8 at local time and zenith angles of ~ 1600 and $\sim 55^\circ$ respectively. These observations were preceded by a violent change in the AE index of 1100γ at 0000–0200 UT on June 6. Surprisingly, in none of these GBR events was there evidence of wave amplification in the wavefield observed over the South Atlantic.

The locations of the most intense amplification ($> 10 \text{ dB}$) events observed for the NAA wavefield in the period 1971 12, December–March 1 are shown in Fig. 1. These all lie in the Southern Hemisphere, slightly to the east of the anomaly, corresponding to amplification of the one-hop whistler mode. The NAA signal throughout this period was almost entirely FSK and was recorded, as for the Ariel III GBR observations, using a minimum reading circuit. An interesting feature of the NAA and GBR one-hop whistler signals, which were observed simultaneously on Ariel IV, was that the GBR signal (FSK) was never amplified in the presence of the more intense NAA signal, however, natural whistlers, at frequencies of 3.2 and 9.6 kHz, did exhibit simultaneous amplification for $\sim 50\%$ of these large amplification events. Apart from a few substorms

Fig. 2 Percentage occurrence of emissions with intensity $> 4.8 \times 10^{-15} \text{ W m}^{-2} \text{ Hz}^{-1}$ at 3.2 kHz, annual electric power consumption (in GWh mile⁻²) and thunderstorm occurrence for the USA $K_p \leq 2+$. — — — —, 1967 Summer (May, June, July). - - - - -, 1967 Autumn (August, September, October). Relative thunderstorm occurrence: summer/autumn = 4 (see text).



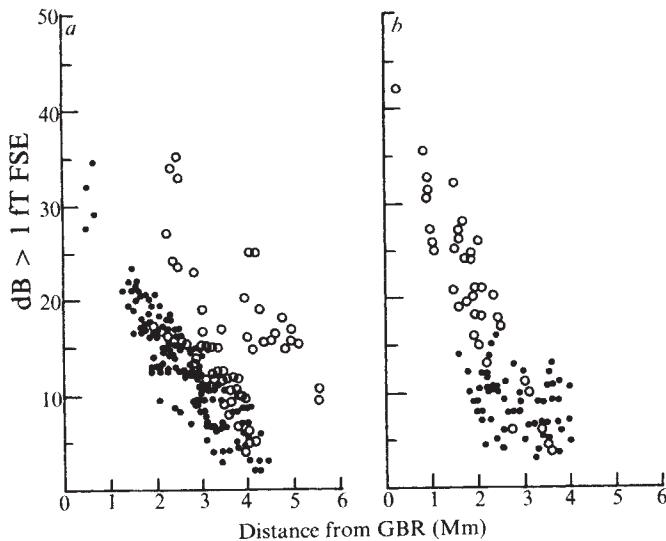


Fig. 3 Daytime amplification of the GBR 16-kHz coded signal over the North Atlantic, 1967 May 12–August 1; all K_p ; $SZA < 90^\circ$. ●, FSK signals; ○, coded (CW) signals. *a*, Azimuthal sector 225–255°E of N; *b*, Azimuthal sector 105–135°E of N.

the NAA signal amplification was associated, as for the GBR wavefield, with solar illumination of the ionosphere at dawn. Further studies of the NAA wavefield by D. J. Davies (personal communication) support this conclusion.

Discussion

From the v.l.f. studies reported above, it is clear that man-made transmissions, particularly the power-line harmonics giving rise to the prominent zone of e.l.f./v.l.f. emission above North America, have an important role in the formation of the electron slot in the magnetosphere. Dungey⁵ was the first to suggest that v.l.f. signals generated on the surface of the Earth might modify the energetic particle population in the magnetosphere by pitch angle diffusion into the loss cone and various authors (see ref. 6) have studied wave particle interaction in the electron slot region. Amplification of the signals in this sector make them much more effective in causing pitch angle diffusion. More direct evidence for wave amplification at mains harmonics, up to 6 kHz, has been found⁷ from ground-based observations in Antarctica. They were able to identify a Canadian aluminium smelting plant as a possible source of some of the signals observed. The signal intensity of the band-limited noise observed on OVI-14 at the magnetic equator lay between 0.03 and 1 m $\gamma\text{Hz}^{-1/2}$ and occurred in the recovery phase of a magnetic storm ($Dst = -120\gamma$). It was sufficient to account for the observed lifetimes of medium and higher energy electrons in the slot. Once the energetic electron population has fallen below the critical flux level indicated by the theory of Kennel and Petschek⁸, then precipitation by a self-generated emission becomes impossible and a wave source is required. Lyons, Thorne and Kennel⁹ have proposed that the electron slot is formed by high order gyroresonant interaction with plasmaspheric e.l.f. hiss, propagating to lower L shells from just within the plasmopause boundary where it is generated. More detailed correlative studies between wave and particle observations are required to ascertain the relative importance of e.l.f. hiss and power line harmonics (first-order cyclotron resonance). The Ariel III and IV evidence for a strong localisation in v.l.f., but not e.l.f. amplification, over the eastern USA suggests that the latter are more important. Theoretical calculations of energetic electron diffusion by the e.l.f. plasmaspheric hiss and power line harmonics indicate that the latter (in first-order resonance) could make an important contribution to diffusion at larger pitch angles where it was found⁹ necessary to invoke higher order resonances for the e.l.f. hiss (Matthews, personal communication). There are also ground-based observations of

band-limited hiss (centred on 5 kHz) which occurs during and after great magnetic disturbances at these invariant latitudes¹⁰.

A recent study¹¹ of the precipitation of energetic electrons from the slot shows that peaked energy spectra, as well as the more normal exponential energy spectra of such electrons, may occur. This peak in the energy spectrum moves to higher energies with decreasing L in such a way that it could be attributed to a first-order cyclotron interaction at a single wave frequency over the range of L sampled. This, of course, is what we might expect to observe for the NAA and/or GBR signal. For the daytime model magnetosphere of Alexander¹² the parallel electron energies for first-order cyclotron resonance at the equator vary from 1 MeV at $L = 1.8$ to 6 keV at $L = 3.0$. Imhof *et al.*¹¹ ascribe the majority of their peaks to first-order resonance at 10 kHz and below (based on the model magnetosphere used) however, lack of data on the actual cold plasma distribution at the time of observation as well as the possibility of a wave-particle interaction at a distance from the equator, make it possible that a higher frequency was involved. The parallel energy, E_R , of the resonant electron is given by

$$E_R = E_c \frac{\omega_H}{\omega} \left(1 - \frac{\omega}{\omega_H} \right)^3$$

where $E_c = B^2/2\mu_0 N$ is the magnetic energy per particle,

$\omega_H = \frac{eB}{m}$ is the electron gyrofrequency (rad s^{-1}), $\omega = 2\pi f$,

B is the local magnetic field, N the local electron density, e the electronic charge, m the electron mass and f the wave frequency (SI units). E_R is clearly very dependent on the value of B .

It has been known for some time (see refs 13–16) that the wave-particle interaction for a ground-based transmitter is a strong function of the duration of the transmitter pulse which causes phase-bunching of the electrons. The preferential amplification of the long duration coded GBR signal, in what is probably the two-hop whistler mode, provides a good example of this. The localisation of the GBR and NAA amplification, slightly to the east of the anomaly, comes from the increase in loss cone and pitch angle anisotropy⁶ of electrons drifting eastwards through the anomaly. Also in this region field aligned structure will help by ducting the whistler wave along the same path as the interacting electrons. The maximum at dawn may be attributed to the decrease in E_c corresponding to an increase in electron density, N , as the cold plasma flows up the field lines from the ionosphere. Thus E_R is decreased and lower energy electrons, which are normally more numerous, drift eastwards into a region where cyclotron resonance becomes possible.

In conclusion, the power line harmonics and other man-made transmissions may, by first-order resonance with the energetic electrons, make a significant contribution to the electron slot between the radiation belts. Thunderstorms over North America also produce e.l.f./v.l.f. wavefields of significant intensity.

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- 1 Lefevre, F., and Bullough, K., *Space Research XIII*, 699–706 (Akademie, Berlin, 1973).
- 2 Koons, H. C., and McPherson, D. A., *J. geophys. Res.*, **77**, 3475–3482 (1972).
- 3 Bullough, K., Hughes, A. R. W., and Kaiser, T. R., *Planet. Space Sci.*, **17**, 363–374 (1969).
- 4 Bullough, K., Sagredo, J. L., Hughes, A. R. W., and Kaiser, T. R., *Space Research XI* (Akademie, Berlin, 1969).
- 5 Dungey, J. W., *Planet. Space Sci.*, **11**, 591–595 (1963).
- 6 Roberts, C. S., *Rev. Geophys. Space Phys.*, **7**, 305–377 (1969).
- 7 Helliwell, R. A., and Katsufurakis, J. P., *J. geophys. Res.*, **79**, 2511–2518 (1974).
- 8 Kennel, C. F., and Petschek, H. E., *J. geophys. Res.*, **71**, 1–28 (1966).
- 9 Lyons, L. R., Thorne, R. M., and Kennel, C. F., *J. geophys. Res.*, **77**, 3455–3474 (1972).
- 10 Hayakawa, M., Tanaka, Y., and Ohtsu, J., *J. atmos. terr. Phys.*, **37**, 517–529 (1975).
- 11 Imhof, W. L., Gaines, E. E., and Reagan, J. B., *J. geophys. Res.*, **79**, 3141–3146 (1974).
- 12 Sagredo, J. L., and Bullough, K., *Planet. Space Sci.*, **20**, 731–746 (1972).
- 13 Helliwell, R. A., *Whistlers and related ionospheric phenomena* (Stanford University Press, California, 1965).
- 14 Helliwell, R. A., *J. geophys. Res.*, **72**, 4773–4790 (1967).
- 15 Nunn, D., *Planet. Space Sci.*, **22**, 349–378 (1974).
- 16 Das, A. C., and Kulkarni, V. H., *Planet. Space Sci.*, **23**, 41–52 (1975).
- 17 Vette, J. I., *Models of the trapped radiation environment*, NASA publ. SP-3024 (1967).

Protein-folding dynamics

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In a discussion of the dynamics of protein folding two limiting models (random-search nucleation and chain propagation, diffusion-collision) are considered. It is suggested that the latter may have the dominant role in many proteins.

THE problem of the folding of a globular protein to its native structure has two parts¹. The first is the static aspect concerned with the elements in the amino acid sequence that provide the information; the second deals with the dynamics of the folding process itself. Clearly, the answer to the first part of the problem is involved in the second (in particular, the stabilities of intermediate structures may be important in selecting the folding paths) and conversely, it is possible, though less likely, that the second affects the first (that is, that the nature of the folding process results in a non-equilibrium structure). Available data indicate that proteins *in vitro* can fold up to their native structure in times from tenths of seconds to minutes in the absence of S-S bridges²; formation of S-S bridges coupled to refolding tends to take longer. This is to be contrasted with the long times required to find the native conformation in a random search of all possible structures (for example, in the simplest model, such a random search would take on the order of 10^{60} yr for a protein consisting of 100 amino acids). Thus, protein folding must make use of more sophisticated search procedures, which, in one way or another, divide a protein molecule into parts such that the information contained in the sequence of each one can be used independently. The simplest solution would be that a protein consisting of 100 amino acids is composed of ten or so approximately equal parts, each of which finds its own unique stable form, and that these parts then come together to form the complete native structure. But it seems from studies of peptides in solution³⁻⁸, as well as approximate energy calculations⁹, that most sequences short enough to be searched through rapidly do not have a folded structure that is sufficiently stable. Thus, alternatives to this simple idea must be examined. Two possible limiting mechanisms for globular protein folding are discussed further below; they are referred to as the random-search nucleation and chain-propagation model and the diffusion-collision model. The latter mechanism is suggested to be more important than the former, though the folding of any particular protein may involve some elements of both limits.

Random-search nucleation and chain propagation

The model utilises a random-search nucleation step as the start of the folding process followed by chain propagation to obtain the native structure. The basic elements of such a mechanism are that first, a portion of the protein is small enough for a random search and yet stable enough to serve as a nucleation unit, and second, that once this unit has the native structure, it makes possible a sequential, essentially independent folding of subsequent amino acids. Such a mechanism was outlined by Wetlaufer¹⁰, and earlier by Levinthal^{11,12}. The process in its simplest version can be formulated by analogy with the treatment of helical dimer formation involving complementary oligonucleotides^{13,14} or helix formation in a polypeptide with only a single nucleation site^{15,16}. Tsong *et al.*^{17,18} have provided a kinetic model for such sequential protein folding and given

kinetic data^{19,20} related to it. The chain-propagation steps are assumed to be analogous to the addition of units in helix formation in polypeptides and, therefore, very fast (that is, of the order of 10^{-6} – 10^{-8} s). Concerning the nucleation step, it is more difficult to make a general statement, although a random search of the conformational alternatives of a minimal nucleus composed of approximately fifteen amino acids would require of the order of milliseconds.

Diffusion-collision

An alternative limiting possibility, the diffusion-collision model, considers the protein molecule to be divided into several parts (microdomains), each short enough for all conformational alternatives to be searched through rapidly. This condition implies that the native secondary structure of each such microdomain, though accessible by random events, is not stable. Consequently, several (two or more) of them have to diffuse together and collide in order to coalesce into a structural entity with the native conformation.

Anfinsen²¹ has discussed the possibility that portions of a protein chain can "flicker" in and out of their native format and serve as nucleation centres coalescing through non-covalent interactions to give the native protein structure; this statement is suggestive of the diffusion-collision model.

To examine the factors determining the kinetics of the model, we consider an example involving two microdomains; this could represent a protein fragment composed of only two parts, or more generally, a portion of an entire protein. Each of the microdomains can be described as being in equilibrium between the native secondary structure and the unfolded random-coil structure, which includes the rest of the available conformational space. If the microdomains formed helices in the native state (as in myoglobin) the helix, random-coil transition would be involved. We assume here that coalescence can occur only if both the partners have the native structure when they collide and that the secondary structural transitions are fast relative to other processes. If the two connected units are regarded as diffusing together from a finite distance to form a stabilised entity with the native conformation, an order of magnitude for the time τ required for the coalescence can be obtained from the radial diffusion behaviour of spherical units. The result is (details will be given elsewhere)

$$\tau \simeq \frac{1}{\beta} \left(\frac{\Delta V}{DA} \right) \quad (1)$$

where ΔV is the volume of the finite diffusion space (the space between two concentric spherical shells); A is the target surface area (the area of the inner shell) whose radius is determined by the sizes of the peptide units; D is the diffusion coefficient and l is a characteristic length (the average of the inner and outer shell radii, the latter being determined by the maximum distance of separation of the two units which are part of the same protein chain). The quantity β accounts for the fact that only a fraction of the microdomains have the native secondary structure when they collide. In a detailed theory, the various parameters involved (that is, ΔV , A , l) would have a direct physical significance, but in this heuristic discussion they are phenomenological in character and only order of magnitude values can be given. With $D \sim 1 \times 10^{-8}$ cm² s⁻¹, a value appropriate for spherical particles with the dimensions of protein fragments, and with the outer shell radius twice the inner one and equal

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to 2×10^{-7} cm (20 Å), equation (1) yields $\tau \sim \beta^{-1} [10^{-7} - 10^{-8}]$ s. To evaluate β , an equilibrium model can be used for simplicity; that is, $\beta = K_1 K_2 / (1 + K_1)(1 + K_2)$ where K_1 and K_2 are the (native)/(random coil) equilibrium constants for the two separated units. This expression ignores the possibility that β could vary as the partners approach and that details of the kinetics might modify the equilibrium result. From the above formulae for τ and β , it follows that for values of K_1 and K_2 equal to $10^{-3} - 10^{-4}$, orders of magnitude equal to the estimated equilibrium constant for helix nucleation in helix-coil transitions²² and the native-random coil equilibrium in protein fragments^{3-8,23}, the folding time for a pair of units is of the order of 0.01 and 10 s. If several units have to come together at one time to form a stable entity and each one has a K_i value less than 10^{-3} , the folding time would be inordinately long. Consequently, a stepwise folding by this mechanism seems most likely; for example, two units coalesce to form a slightly more stable entity which in turn collides with a third entity, and so on. In this way the rate of the process would increase as the folding advanced, as expected for a cooperative transition.

Variation of τ with temperature is expected due to both D and β . The Einstein-Stokes expression for D approximately doubles in size between 10 °C and 40 °C. The dependence of β on temperature is difficult to estimate since it is determined by a balance between enthalpic and entropic (for example, hydrophobic) terms. Some temperature variation has been observed in the renaturation rate of acidified staphylococcal nuclease²⁴. The results are not, however, sufficiently detailed to relate to the model discussed here.

Several refinements would have to be introduced into the model to make calculations of more than order-of-magnitude validity. These include a more detailed consideration of the size, shape and connections of the colliding segments, the possibility of the existence of an activation barrier to coalescence, the requirement for rotational as well as translational motion to obtain the correct position for a stable interaction, and the consideration of diffusion in the presence of a folding/unfolding process with the same time scale. Most of these effects are expected to increase the time required, though changes by several orders of magnitude seem unlikely except for the significant activation barrier.

Implications of the models

Two limiting mechanisms that could be involved in the folding of proteins to their native structure have been discussed briefly and their dynamics considered. The true folding process of a real protein may well involve elements of both extremes. Some amino acids would undoubtedly take up the native format in a sequential, rather fast (μ s) manner whether the primary process were a random-search nucleation or a diffusion-collision step or a combination of the two. It seems, however, that folding an entire protein of 100 or more amino acids by a single random-search nucleation plus chain-propagation mechanism is not feasible. The difficulty is that rapid propagation after nucleation requires that the laying down of each successive amino acid or small group of amino acids be essentially independent of the conformation of the rest of the unfolded molecule. Although this may be a good approximation for helix formation, it seems unlikely for the complicated structure of globular proteins. If each propagation step requires a search through a significant fraction of the conformations of the uncoiled portion of the protein chain, the basic folding problem, albeit in a slightly reduced form, remains unsolved after nucleation. It has been noted in analyses of structural data that many larger proteins are composed of domains, each of which could fold almost independently⁶. Since these domains are composed of 50–150 amino acids, they are too large to serve as primary nucleation centres; instead they each raise the same folding problem as the entire molecule.

In contrast to the difficulties that are found with the random-search chain-propagation model, available structural and other data suggest that the diffusion-collision model can serve as

the basis for a mechanism of protein folding. Several recent studies have shown that globular proteins have about one-third of their residues involved in chain-reversal turns^{25–27}. Since each turn is normally composed of four residues (by the criteria used in the analyses), this result implies that a protein is on average composed of segments eight to ten residues between turns. Thus, it seems natural to suggest that these segments form the primary diffusing units in the diffusion-collision mechanism. The entire folding process would, as described above, involve a series of such diffusion-collision steps. These might have to follow a unique order to yield the native structure (single correct pathway); alternatively, different sequences of diffusion-collision steps might be possible. Further, particularly in the larger proteins, there may be several regions (the domains) that are individually fixed in their native structure by a set of diffusion-collision steps and finally come together to form the completed native protein molecule.

The diffusion-collision mechanism would be most effective if the individual segments (the diffusing units or microdomains) were relatively unlikely to bend significantly; that is, that they can be regarded as statistically rigid rods in the diffusion process so as to minimise entanglement problems. Empirical correlations tend to support this view^{25–27}, as does the model calculation of Levitt and Warshel²⁸. Some regions which have a very well defined secondary structure (for example, α helices) could support more residues per segment; significant deviations from the average of eight to ten residues do occur, although a detailed correlation with the folding mechanism has not been made.

The motion emphasised here is essentially diffusive, and the attractive forces that stabilise the coalesced microdomains are short range; that is, until two groups are sufficiently close to prevent water molecules from being between them, no hydrophobic attraction can exist. Moreover, any directing forces provided by the connecting elements are likely to be damped by hydrodynamic effects. Once the segments have diffused together, the balance between hydrophobic attraction and van der Waals' repulsion will determine whether or not coalescence can occur in a given collision. This requires that segments have the correct secondary structure (including the side-chain orientation)²⁹; otherwise a significant repulsive barrier would be expected. Consequently, the parameter β , which determines the fraction of the time that the correct secondary structure exists, has a fundamental role in the effective time constant of the diffusion-collision mechanism (equation (1)). It would be very useful to have experimental measurements or theoretical estimates of β for protein fragments that are likely candidates for the microdomains involved in the diffusion-collision process. These might be pairs of helices, pairs of peptide chain segments that can form a β sheet, or an α -helix plus β -sheet structure (ref. 30 and references therein); in the present context, a β sheet is regarded as an element of tertiary structure because it is formed by the coming together of two elements, each with the appropriate extended secondary structure. Such fragments might be made available for study either by cleavage of the native protein or by direct synthesis. Comparisons can be made also with the available equilibrium constants for the (native)/(random coil) transition of complete proteins³¹. Kinetic measurements for the renaturation of fragment-type systems are important as well; some data are available for the *Staphylococcus* nuclease peptides³². Possible model systems include glucagon (which has a random coil structure in dilute solution, but at high concentration forms a trimer composed mainly of α helices)³³, and the polypeptides that form β -sheet networks³³.

Incorrectly folded intermediates have been suggested on the basis of a kinetic analysis by Ikai and Tanford³⁴. Creighton³⁵ has trapped intermediates with incorrect disulphide bonds in his study of the folding of the basic pancreatic trypsin inhibitor; both the correct and incorrect early intermediates (one disulphide bond) seem to be correlated with stabilising secondary structural elements of the type mentioned above^{25–27,30}. Such incorrect folding might be expected if the diffusion-collision

mechanism has an important role; for example, segments that are not together in the native structure could collide and form intermediates which are relatively unstable because of improper non-bonded contacts.

Two theoretical attempts^{28,36} to fold a protein have been made as a means of investigating the static part of the problem. One of these, by Ptitsyn and Rashin³⁶, considered myoglobin as composed of stable helices and, on the basis of a simple model for hydrophobic interactions between the helices, compared different paths to the most likely folded structure, a rough approximation to the native conformation. This type of static analysis is certainly compatible with the diffusion-collision mechanism; in a corresponding kinetic model, the helix-coil equilibria of the different helices would have to be taken into account. Levitt and Warshel²⁸ have tried to obtain the native structure of the basic pancreatic trypsin inhibitor by energy minimisation. They began with a simplified model of the extended polypeptide chain that can bend easily at only a few amino acids. The protein is thereby divided into segments that simplify the folding process as in the diffusion-collision mechanism. An important additional element in their calculation is that the assumed balance between attractive and repulsive forces is such that the colliding segments do not need to have the correct secondary structure for coalescence to occur. If this were valid for the actual folding process, the kinetic behaviour would be significantly different from that described above, since the parameter β would not appear in equation (1). There would result a rapid diffusion step leading to an approximate structure, followed by a slower process involving local readjustments to the native structure. Most likely, the situation in proteins is intermediate, with some probability of coalescence even when the colliding segments have an only approximately correct secondary structure.

Many of the data used in published discussions of protein folding are obtained in studies of unfolding². This raises the question of whether the pathways for the two processes are the same. Since diffusion of polypeptide segments over the distances involved requires less than a microsecond, it is hard to account for the observed unfolding times, which are of the order of seconds, by assuming a simple reversal of the folding process. Consequently, it seems necessary to introduce a slow random event (for example, nucleation of open regions) in the unfolding pathway that is different from that in folding. An alternative

possibility, which has not been considered in detail here, is that both processes are limited by a high activation barrier involving the same intermediates.

The analysis given here shows that the information required for a complete description of the dynamics of protein folding is not yet available. We hope, however, that our discussion of different limiting mechanisms will stimulate others to perform the experiments necessary to clarify the processes involved.

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- ¹ Anfinsen, C. B., and Scheraga, H. A., *Adv. Protein Chem.*, **29**, 205-300 (1975).
- ² Baldwin, R. L., *A. Rev. Biochem.*, **44**, 453-475 (1975).
- ³ Brown, J. E., and Klee, W. A., *Biochemistry*, **10**, 470-476 (1971).
- ⁴ Eppand, R. M., *J. Biol. Chem.*, **247**, 2132-2138 (1972).
- ⁵ Hammes, G. C., and Schullery, S. E., *Biochemistry*, **7**, 3882-3887 (1968).
- ⁶ Gratzner, W. B., and Beaven, G. H., *J. Biol. Chem.*, **244**, 6675-6679 (1969).
- ⁷ Panippan, B., and Gratzner, W. B., *Eur. J. Biochem.*, **45**, 547-553 (1974).
- ⁸ Sasaki, K., Dockerrill, S., Adamiak, D. A., Tickle, I. J., and Blundell, T., *Nature*, **257**, 751-757 (1975).
- ⁹ Ponnuswamy, P. K., Warme, P. K., and Scheraga, H. A., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 830-833 (1973).
- ¹⁰ Wetlauffer, D. B., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 697-701 (1973).
- ¹¹ Levinthal, C., *J. Chim. phys.*, **65**, 44-45 (1968).
- ¹² Levinthal, C., *Scient. Am.*, **214** (6), 42-52 (1966).
- ¹³ Craig, M. E., Crothers, D. M., and Doty, P. M., *J. molec. Biol.*, **62**, 383-401 (1971).
- ¹⁴ Porschke, D., and Eigen, M., *J. molec. Biol.*, **62**, 361-381 (1971).
- ¹⁵ Schwarz, G., *J. molec. Biol.*, **11**, 64-77 (1965).
- ¹⁶ Schwarz, G., *Biopolymers*, **6**, 873-893 (1968).
- ¹⁷ Tsong, T. Y., Baldwin, R. L., and McPhie, P., *J. molec. Biol.*, **63**, 453-475 (1972).
- ¹⁸ Tsong, T. Y., Baldwin, R. L., and Elson, E. L., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1809-1812 (1972).
- ¹⁹ Garel, J.-R., and Baldwin, R. L., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3347-3351 (1973).
- ²⁰ Pohl, F. M., *Angew. Chem. int. Ed.*, **11**, 894-906 (1972).
- ²¹ Anfinsen, C. B., *Science*, **181**, 223-230 (1973).
- ²² Zimm, B. K., and Bragg, J. K., *J. chem. Phys.*, **31**, 526-535 (1959).
- ²³ Sachs, D. H., Schechter, A. N., Eastlake, A., and Anfinsen, C. B., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3790-3794 (1972).
- ²⁴ Epstein, H. F., Schechter, A. N., Chen, R. F., and Anfinsen, C. B., *J. molec. Biol.*, **60**, 499-508 (1971).
- ²⁵ Lewis, P. N., Momany, F. A., and Scheraga, H. A., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2293-2297 (1971).
- ²⁶ Crawford, J. L., Lipscomb, W. N., and Schellman, L. G., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 538-542 (1973).
- ²⁷ Chou, P. Y., and Fasman, G. D., *Biochemistry*, **13**, 222-245 (1974).
- ²⁸ Levitt, M., and Warshel, A., *Nature*, **253**, 694-698 (1975).
- ²⁹ Gelin, B., and Karplus, M., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 2002-2006 (1975).
- ³⁰ Nagano, K., *J. molec. Biol.*, **84**, 337-372 (1974).
- ³¹ Furie, B., Schechter, A. N., Sachs, D. H., and Anfinsen, C. B., *J. molec. Biol.*, **92**, 497-506 (1975).
- ³² Light, A., Taniuchi, H., and Chen, R. F., *J. Biol. Chem.*, **249**, 2285-2293 (1974).
- ³³ Snell, C. R., and Fasman, G. D., *Biochemistry*, **12**, 1017-1025 (1973).
- ³⁴ Ikai, A., and Tanford, C., *Nature*, **230**, 100-102 (1971).
- ³⁵ Creighton, T. E., *J. molec. Biol.*, **87**, 603-624 (1974).
- ³⁶ Ptitsyn, O. B., and Rashin, A. A., *Biophys. Chem.*, **3**, 1-20 (1975).

Mechanism localisation and control of restriction cleavage of phage T4 and λ chromosomes *in vivo*

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The primary action of restriction endonuclease, cleaving infecting DNA, has been demonstrated in vivo. This primary cleavage is followed rapidly by hydrolysis of the cleaved DNA at its newly exposed termini. Infecting viruses can inactivate cytoplasmic and membrane restriction endonucleases to prevent cleavage of unmodified DNA replicas.

THE utility of restriction endonucleases as a tool in biological research and their potential in genetic engineering have aroused much interest in these enzymes^{1,2}. Considerable information exists on the mechanism of action of restriction enzymes *in vitro*³⁻⁵. Much less is known about their function within cells. Recognition *in vitro* is somewhat ambiguous⁶ and in fact some

chromosomes which are cleaved by isolated restriction endonucleases *in vitro* are not restricted by bacteria containing these endonucleases *in vivo*^{7,8}. Restriction endonucleases normally recognise a specific short nucleotide sequence in double-stranded DNA. There are two major classes of purified restriction endonucleases, defined as specific (class II) and nonspecific (class I), depending on their cleavage specificity *in vitro*². Class II enzymes cleave the DNA at the restriction site by opposite or staggered nicks⁹⁻¹³. This distinction can be reduced by altering incubation conditions⁶. In addition, single-stranded DNA can be cleaved¹⁴ in certain conditions. Class I enzymes can cleave at any place up to a few hundred nucleotides away from the recognition site. We wonder which of these variations reflect the variety of mechanisms of action of these enzymes within the

Table 1 Restriction and breakdown of T4 *glu*⁻ DNA and T4 2⁻ DNA in various hosts

<i>E. coli</i> strain	<i>rgl</i>	exo V	exo I	T4 phage	Plating efficiency	% TCA-soluble DNA
K	—	+	+	<i>glu</i> ⁻	1	3
K	—	+	+	2 ⁻	1 × 10 ⁻⁴	40
B	+	+	+	<i>glu</i> ⁻	1 × 10 ⁻⁵	42
B	+	+	+	2 ⁻	5 × 10 ⁻⁶	48
W3110	+	—	+	<i>glu</i> ⁻	2 × 10 ⁻⁴	8
W3110	+	—	+	2 ⁻	0.7	8
JC7623	+	—	—	<i>glu</i> ⁻	2 × 10 ⁻⁴	5
JC7623	+	—	—	2 ⁻	1	6

Standard phage techniques were those of Adams³¹ and other techniques and strains used were described by Silverstein and Goldberg^{29,30}. DNA breakdown is determined as the TCA-soluble fraction of DNA. *Glu*⁻ phage were T4 α gt⁵⁷βgt¹⁴ grown on *E. coli* K *rgl*⁻, 2⁻ were T4 am N51 grown on *E. coli* B. The data given are not corrected for absorption which was 85–90% for both 2⁻ and *glu*⁻ phage.

cell and which are merely artefacts of the *in vitro* environment.

Recognition sites in the DNA may be modified specifically (for example, methylation of *E. coli* DNA and λ DNA^{15,16} or glucosylation of T-even DNA^{17–19}), rendering the DNA insensitive to the appropriate restriction enzyme. *In vitro*, the action of restriction enzyme is usually studied by observing the cleavage of an intact viral chromosome into large fragments. For example, phage λ DNA is cleaved *in vitro* at five sites by *fi*⁺ R-factor-coded restriction endonuclease (endo R. *EcoRI*²⁰). *In vivo*, the cleavage by restriction endonucleases is followed by extensive hydrolysis by nonspecific nucleases^{21–25}, resulting finally in acid-soluble fragments^{12,26}. We call this hydrolysis DNA breakdown, to distinguish it from the initial cleavage. Breakdown of unmodified λ DNA in *E. coli* K depends on the function of the bacterial *rec BC* locus. Growth of unmodified phage is restricted in *rec BC*⁻ hosts even though DNA breakdown does not occur²⁷. In considering the possible biological mechanism of action of restriction enzymes in the cell, we were surprised that large DNA fragments have never been demonstrated *in vivo* as primary products of restriction endonuclease cleavage. In this report we try to clarify the relationship between the initial cleavage by restriction endonucleases and the subsequent breakdown of the cleaved fragments by exonuclease V (exo V is specified genetically by *rec BC*)²⁸ *in vivo*. Using *rec BC*⁻ bacteria, we were able to prevent degradation of the fragments cleaved from the unmodified T4 or λ phage chromosome by the cellular restriction endonucleases. Therefore we were able to demonstrate that these large fragments are intermediates in the restriction process *in vivo*, as postulated after *in vitro* experiments. We were also able to show that different restriction endonucleases which are thought to be localised in the cytoplasm or in the membrane can be inactivated in different ways by the infecting phage.

Role of cleavage and breakdown in restriction of T4

A phage T4 amber mutant in gene 2 produces particles of normal appearance in non-permissive *Su*⁻ hosts. Though they adsorb and eject DNA normally, they do not produce progeny on *Su*⁻ or *Su*⁺ bacteria unless the bacteria lack *exo V*^{29,30}. Table 1 compares the restriction (plating efficiency) of phage particles defective in gene 2 (2⁻) and non-glucosylated (*glu*⁻)

phage and the breakdown (% of trichloroacetic acid (TCA)-soluble DNA) of their DNA after infection. In the presence of *exo V* both 2⁻ and *glu*⁻ infecting chromosomes are broken down, but in the absence of *exo V* they are not. In the presence of *rgl* (the restriction systems for non-glucosylated T-even phages) growth of 2⁻ phage is not restricted, whereas growth of *glu*⁻ phage is. In *rgl*⁻ hosts, with or without *exo V*, *glu*⁻ DNA is not broken down nor are *glu*⁻ phages restricted.

These findings can be explained by the model illustrated in Fig. 1. T4 gene 2 specifies a protein (P2) which seems to protect

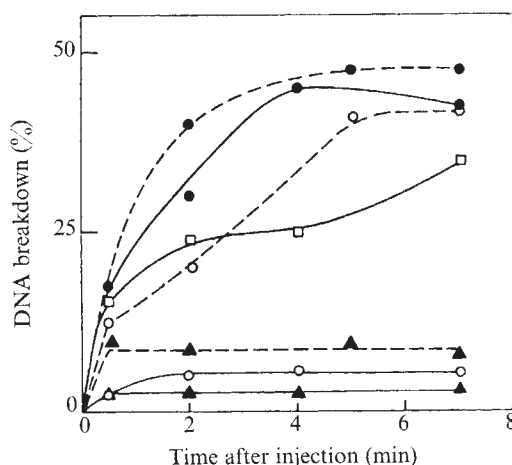
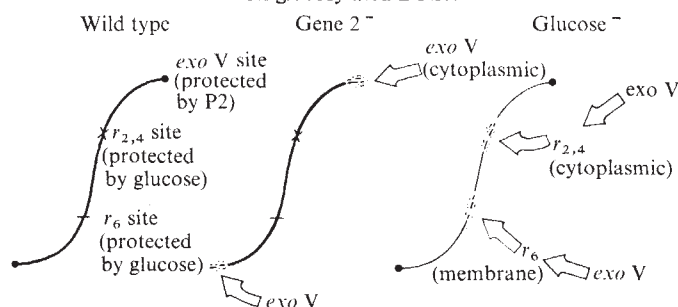


Fig. 2 Kinetics of breakdown of T4 *glu*⁻ DNA and T4 2. *Su*⁻ DNA in various hosts. Experimental details are as described in Table 1. ●—●, *glu*⁻ T4 in *rgl*⁺ *exo V*⁺ *exo I*⁺ (B); ▲—▲, *glu*⁻ T4 in *rgl*⁻ *exo V*⁺ *exo I*⁺ (K *rgl*⁻); ○—○, *glu*⁻ T4 in *rgl*⁺ *exo V*⁻ *exo I*⁻ (JC7623); □—□, *glu*⁻ T4 in *rgl*⁺ *exo V*⁺ *exo I*⁻ (W3110 *sbcB*); ●—●, T4 2.*Su*⁻ in *rgl*⁺ *exo V*⁺ *exo I*⁺ (B); ▲—▲, T4 2.*Su*⁻ in *rgl*⁺ *exo V*⁻ *exo I*⁻ (JC7623); ○—○, T4 2.*Su*⁻ in *rgl*⁺ *exo V*⁺ *exo I*⁻ (W3110 *sbcB*).

the end(s) of the entering phage genome from *exo V*. Modification of the DNA by glucosylation protects the entering genome from the *rgl* endonucleases. In the absence of P2, *exo V* would digest the entering DNA exonucleolytically. This action would thus be directly responsible for both the breakdown of part of the genome and the consequent restriction of the phage. The presence of P2 on the unmodified genome could protect the natural termini from *exo V*, but could not protect newly exposed ends created by endonucleolytic action of the *rgl* systems. Thus, as Table 1 shows, restriction of T4 *glu*⁻ phage would be a direct result of cleavage of the genome by the *rgl* systems, whereas breakdown would be a secondary result of *exo V* action on the newly formed unprotected ends.

According to this model, it should be possible to demonstrate *in vivo* the initial cleavage products of unmodified DNA by infection of cells lacking *exo V*. To demonstrate such fragments we had first to determine the proper strains and appropriate time after infection to extract the DNA. Figure 2 shows that when *exo V* cannot digest the DNA, there is little acid-soluble material produced up to 7 min. This is the case even up to 25 min (unpublished). In cases where breakdown occurs it is usually complete by 5 min after infection. When

Fig. 1 A model for the mechanism of restriction of 2⁻ and *glu*⁻ T4 phage by endonucleolytic cleavage and exonucleolytic breakdown of DNA. Thick line, glucosylated DNA; thin line, non-glucosylated DNA.



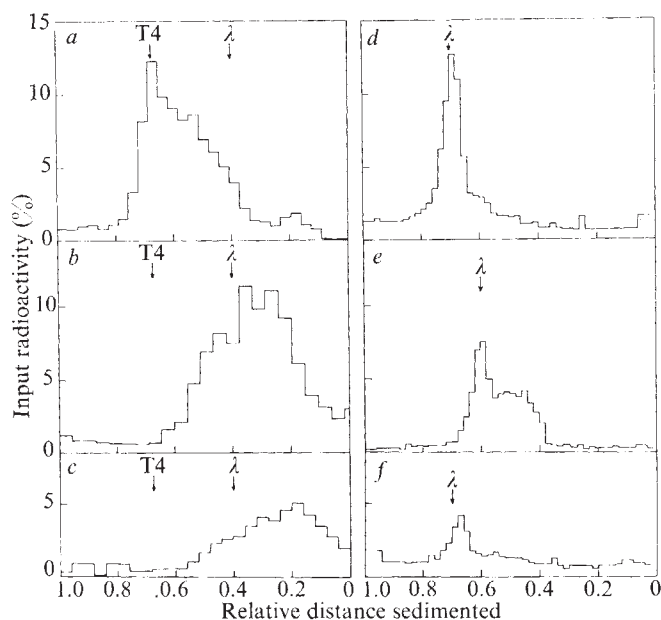


Fig. 3 Demonstration of primary cleavage products of *rgl* and *rK* restriction *in vivo*. *a, b* and *c*: T4, *rgl* restriction. Cells (5×10^8 per ml) were infected at 37°C with two or three ^{32}P -glu $^-$ T4 phage each. After 5 min aeration at 37°C cells were lysed and 0.2-ml samples were layered on to 4.5 ml 5%-20% alkaline sucrose gradients³². Marker phage (^3H - λ) was added to the infected cell lysate after addition of sarkosyl. The DNA was sedimented for 100 min at $147,000g$ at 20°C , fractionated by dripping on to Whatman scintillation pads, and washed with cold TCA. The 5% cold TCA removed acid-soluble nucleotides and therefore some of the graphs show less than 100% recovery. The calculated position³³ of T4 DNA shown in each graph was checked experimentally on several occasions. *a*, *rgl* $^+$ exo V $^+$ exo I $^+$ (K *rgl* $^-$); *b*, *rgl* $^+$ exo V $^-$ exo I $^-$ (JC 7623); *c*, *rgl* $^+$ exo V $^+$ exo I $^+$ (B). *d, e* and *f*: λ , *rK* restriction. Cells (5×10^8 per ml in 0.01 M Tris buffer, pH 7.4, 0.001 M MgSO_4) were infected with two or three ^{32}P - λC_{1857} C_{600} *rK* $^-$ *mK* $^-$ per cell. Cells and phage were prepared as described before³⁴. After 5 min of aeration at 37°C cells were lysed and analysed in alkaline gradients as described above except they were run at $192,000g$ for 120 min. Adsorption of λ was 85-90% at 5 min after infection for all cells. *d*, *rK* $^-$ *mK* $^-$ exo V $^+$ (C600); *e*, *rK* $^+$ *mK* $^+$ exo V $^-$ (JC 7623); *f*, *rK* $^+$ *mK* $^+$ exo V $^+$ (QL).

exo V digests DNA in the absence of exo I, the production of acid-soluble fragments is delayed to some extent (Fig. 2). Thus the presence of exo I makes, at most, a transient difference in acid solubility and does not affect cleavage by *rgl*.

The products of cleavage and subsequent breakdown were analysed by sucrose gradient centrifugation (Figs 3 and 4). In the absence of exo V the *rgl* systems cut each DNA strand in only a few places and there is but little breakdown of DNA to small acid-soluble or insoluble fragments (Fig. 3b). DNA strands from non-glucosylated T4, are intact after infection of a host which contains exo V but lacks the *rgl* systems (Fig. 3a). Hosts with both exo V and *rgl* systems yield DNA strands which are much shorter on the average than in exo V $^-$ *rgl* $^+$ hosts, and much of the DNA is lost when the filters are washed with TCA since it is TCA-soluble (Fig. 3c). Neutral gradients give results consistent with this interpretation (data not shown), since on *rgl* $^+$ exo V $^-$ hosts the entering DNA is cleaved and sediments as a few peaks, the amplitude of which is greatly reduced in *rgl* $^+$ exo V $^+$ hosts. Thus we have been able to demonstrate the *in vivo* *rgl* cleavage products of unmodified T4 genomes. This cleavage prevents T4 growth even in the absence of breakdown by exo V (Table 1).

The model in Fig. 1 predicts a very different result for the degradation of the 2 $^-$ genome. If restriction is a direct result of exonucleolytic attack, the large DNA fragments should get progressively shorter, and the small single-stranded excised pieces should be produced directly without intermediate sized fragments. Figure 4 shows the time course of degradation of

parental 2 $^-$ DNA in *rgl* $^+$ exo V $^+$ and *rgl* $^+$ exo V $^-$ hosts. The DNA molecules are gradually shortened, and the excised single-stranded fragments are converted directly to small acid-soluble or insoluble pieces in exo V $^+$ cells. This is consistent with the action *in vitro* of exo V, which degrades double-stranded DNA directly into single-stranded fragments one to several hundred nucleotides long³⁵. Since 2 $^-$ DNA is fully glucosylated it is not cleaved by the *rgl* system. Figure 4 shows a small amount of short acid-insoluble oligonucleotides sedimenting between 0 and 15% from the top of the gradient. The amount of this material is roughly constant from 1 to 4 min after infection while the total amount of breakdown products as measured by acid solubility rises rapidly (30%, 40%, 45% and 48% in 1, 2, 3 and 4 min, respectively). Thus it seems that exo V, *in vivo*, hydrolyses DNA into oligonucleotides up to 500-600 units long (remaining at the top of the gradients), which may well be the initial products of degradation. These are then further degraded to acid-soluble material by exo V, as has been shown *in vitro*³⁵. This secondary degradation to mononucleotides may be aided by exo I which can rapidly hydrolyse short single-stranded DNA fragments of glucosylated as well as non-glucosylated DNA to mononucleotides. This idea is consistent with our findings (Fig. 2) that in exo V $^+$ exo I $^-$ cells, the breakdown of glu $^-$ and 2 $^-$ DNA is slower than in exo V $^+$ exo I $^+$ strains. The final ratio of acid-soluble to insoluble

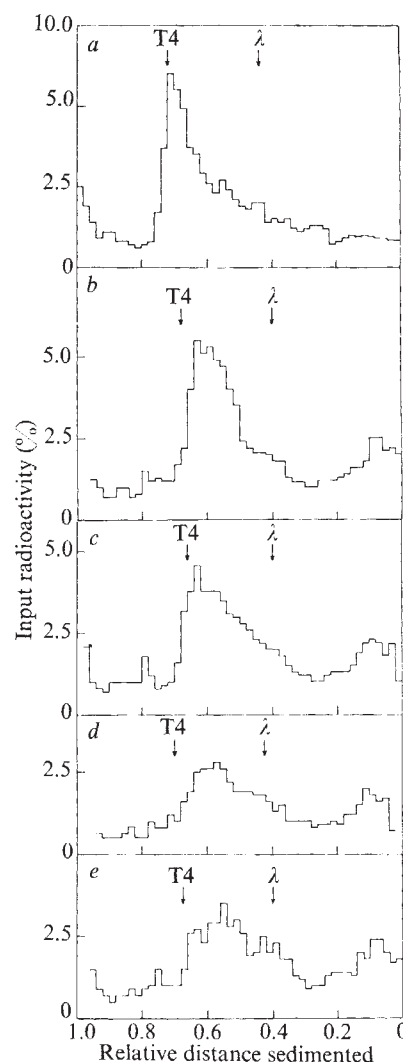


Fig. 4 Kinetics of digestion of T4 2 $^-$ DNA *in vivo*. Experimental details were as described in Fig. 3. Aliquots of infected cells were lysed at the appropriate times and 0.2-ml samples were layered on the alkaline sucrose gradients. *a*, exo V $^-$ exo I $^-$ (JC 7623) cells lysed 5 min after infection. *b, c, d*, and *e*, exo V $^+$ exo I $^+$ (B) cells lysed 1, 2, 3 and 4 min, respectively, after infection.

material may be due in part to reincorporation of acid-soluble products into acid-insoluble material.

Simmon and Lederberg²⁷ showed that breakdown of unmodified λ DNA *in vivo* is aided by exo V in restricting hosts, and is independent of other nucleases, like exo I. Based on our data on glu^- T4 restriction, our model (Fig. 1) explains the mechanism of restriction of T4 *in vivo* independently of exo V. According to this model, unmodified λ should be restricted even in exo V⁻ hosts. Restriction of growth of unmodified λ has already been shown²⁷. In restricting exo V⁺ cells, at low multiplicities of infection, unmodified λ DNA is broken down to acid-soluble nucleotides (Fig. 3f). Only about 10% of the DNA sediments along with the marker DNA, and no high molecular weight intermediates are found in these conditions. Analysis of restriction products in similar conditions in a restricting exo V⁻ host is shown in Fig. 3e. About 75–80% of the λ strands are cut into large fragments of 0.4–0.9 the size of λ . This agrees with the *in vitro* results^{9,12} first shown by Meselson and Yuan. The rest of the DNA strands cosediment with whole λ marker strands. Only about 5% of the DNA is degraded to acid-soluble fragments. This indicates that the absence of large intermediates in exo V⁺ cells is due to the secondary action of exo V. The efficiency of restriction is more or less the same in restricting hosts whether or not exo V is present.

Localisation of r_6 and $r_{2,4}$

Revel³⁶ showed that the *rgl* function is controlled by two genes: r_6 which restricts all glu^- T-even phages, and $r_{2,4}$ which restricts only glu^- T2 and T4. Fukasawa²² proposed that the *rgl*

system is a membrane function since modified (glu^+) parental DNA could escape restriction in an *rgl*⁺ UDPG pyrophosphorylase⁻ host allowing the unmodified (glu^-) progeny DNA to replicate and mature. Fleischman and Richardson³⁷, however, on the basis of *in vitro* experiments showed that *rgl* acts on replicating DNA. Though their data seem to support a cytoplasmic site for $r_{2,4}$, they point out it may nevertheless be membrane bound. Hewlett and Mathews³⁸ reinforced the proposal of Fukasawa by showing that superinfecting glu^- T6 is not restricted after T4 *imm*⁻ primary infection. (The *imm*⁻ mutation prevents exclusion and breakdown of superinfecting T4 chromosomes). They suggest that the mere attachment of the primary phage inhibits *rgl* functions since the successful passage of the superinfecting glu^- T6 does not depend on primary phage protein synthesis. Silverstein and Goldberg³⁹ found that successful superinfection of glu^- T4, in contrast to glu^- T6, requires protein synthesis by the primary phage. They suggested that, although r_6 may indeed be localised in the membrane, as Hewlett and Mathews³⁸ concluded, $r_{2,4}$ is probably cytoplasmic, as suggested by the experiments of Fleischman and Richardson³⁷. On the basis of these experiments and those reported below, we suggest that r_6 is a membrane function whereas $r_{2,4}$ is probably cytoplasmic.

Table 2 shows that superinfecting glu^- DNA is broken down in $r_{2,4}^+$ cells only when expression of the primary infecting phage is prevented. Expression was prevented either by addition of chloramphenicol before primary infection, or by using heavily ultraviolet-irradiated primary infecting phage, which cannot express many genetic functions after infection (for

Table 2 Localisation of restriction functions $r_{2,4}$ and r_6

Bacterial strains	Chloramphenicol	Primary phage	Superinfecting phage	% DNA breakdown of superinfecting phage
$r_6^- r_{2,4}^-$	—	T4 <i>imm</i> ⁻	T4 <i>glu</i> ⁻	3
	—	T4 <i>imm</i> ⁻	T6 <i>glu</i> ⁻	4
	+	T4 <i>imm</i> ⁻	T4 <i>glu</i> ⁻	3
	+	T4 <i>imm</i> ⁻	T6 <i>glu</i> ⁻	3
	—	UV.T4	T4 <i>glu</i> ⁻	14
	—	UV.T4	T6 <i>glu</i> ⁻	9
$r_6^+ r_{2,4}^-$	—	T4 <i>imm</i> ⁻	T4 <i>glu</i> ⁻	4
	—	T4 <i>imm</i> ⁻	T6 <i>glu</i> ⁻	2
	+	T4 <i>imm</i> ⁻	T4 <i>glu</i> ⁻	3
	+	T4 <i>imm</i> ⁻	T6 <i>glu</i> ⁻	8
	—	UV.T4	T4 <i>glu</i> ⁻	16
	—	UV.T4	T6 <i>glu</i> ⁻	6
$r_6^- r_{2,4}^+$	—	T4 <i>imm</i> ⁻	T4 <i>glu</i> ⁻	7
	—	T4 <i>imm</i> ⁻	T6 <i>glu</i> ⁻	3
	+	T4 <i>imm</i> ⁻	T4 <i>glu</i> ⁻	28
	+	T4 <i>imm</i> ⁻	T6 <i>glu</i> ⁻	3
	—	UV.T4	T4 <i>glu</i> ⁻	43
	—	UV.T4	T6 <i>glu</i> ⁻	12
$r_6^+ r_{2,4}^+$	—	T4 <i>imm</i> ⁻	T4 <i>glu</i> ⁻	5
	—	T4 <i>imm</i> ⁻	T6 <i>glu</i> ⁻	3
	+	T4 <i>imm</i> ⁻	T4 <i>glu</i> ⁻	35
	+	T4 <i>imm</i> ⁻	T6 <i>glu</i> ⁻	3
	—	UV.T4	T4 <i>glu</i> ⁻	35
	—	UV.T4	T6 <i>glu</i> ⁻	9

The log phase cultures were collected and resuspended at a cell density of 5×10^8 cells per ml in broth and three T4 *imm*2 phage were added per bacterium. After 5 min two or three ^{32}P - glu^- T4 were added per bacterium. Chloramphenicol was added ($100 \mu\text{g ml}^{-1}$) when required 3 min before addition of primary phage. Adsorption and DNA breakdown were assayed 5 min after addition of superinfecting phage. Wild-type T4 phage (10^{11} per ml) were irradiated in washing fluid²⁹ for 10 min to a survival of $<0.001\%$. These UV.T4, killed cells at an efficiency of 90% of unirradiated phage. UV.T4 particles were used for primary infection at a multiplicity of 4–5. ^{32}P -T6agt^{am10am16} grown on K *rgl*⁻ Su⁻ were used for superinfection experiments.

example, immunity to superinfection, T4-induced endonuclease³⁹ and anti-exo V, Table 2). Superinfecting glu^- T6 DNA, however, is protected from breakdown in r_6^+ (with or without $r_{2,4}$) cells by the primary infecting phage, even when expression of the primary phage is prevented by chloramphenicol or ultraviolet irradiation.

Controls of restriction systems

The breakdown of superinfecting glu^- DNA by exo V^+ in an $r_{2,4}^+$ exo V^+ cell, in the absence of primary phage expression, implies previous cleavage of the superinfecting DNA by an rgl enzyme (Fig. 1). The lack of breakdown of the superinfecting glu^- DNA in an $r_{2,4}^+$ exo V^+ cell, after expression of the primary phage, is to be expected (whether or not the rgl system is working) as the result of the expression of an exo V inactivating activity by the primary infecting phage^{40,41}. We have shown that the glu^- superinfecting DNA is not cleaved in $r_{2,4}^+$ cells⁴². Therefore the primary infecting phage must code for an anti- $r_{2,4}$ activity which inactivates $r_{2,4}$ after infection. Thus, the reason that unmodified progeny T-even DNA can replicate from a modified parental template and mature in $r_6^+ r_{2,4}^+$ hosts (where unmodified parental DNA is normally restricted) is because the early interaction of the infecting phage particle with the host surface inactivates r_6 , and subsequent expression of the unrestricted parental genomes leads to a protein which prevents $r_{2,4}$ action on the unmodified progeny DNA.

It is interesting in this respect that unmodified λ is also not restricted by the cytoplasmic r_K in the presence of the sbcA mutation²⁷. It would be interesting to know if the sbcA mutation leads to inactivation of r_K or possibly whether it prevents the action of r_K on the unmodified λ chromosome.

There is a series of proteins produced by the phage which protects its DNA at various stages of infection (see refs 43 and 44). The phage coat itself is a group of proteins protecting the DNA during transfer from one host to another. The gene 2 protein of T4 protects the DNA from exo V when entering the host. We predict that analogous proteins will be found on other double-stranded linear DNA containing phage possibly gene 2 of phage T7⁴⁴, especially since many of them also make a protein which inactivates exo V (refs 40 and 41). We also think that a protein attached to the DNA terminus may have other functions in morphogenesis, injection and expression, similar to the "pilot" protein of single-stranded DNA phage⁴⁵. T4 phage also codes for an activity which blocks $r_{2,4}$ restriction endonuclease⁴². The presence of 20–25% of intact λ strands after infection of r_K^+ exo V^- cells at a multiplicity of 2–3 may reflect unadsorbed and unrejected DNA, but it may also reflect protection of some late entering chromosome from r_K by an anti r_K -activity synthesised from the large cleavage fragments of the first genome which entered.

The restriction-modification system seems to serve as a defence and speciation mechanism which can alter the genetic effectiveness of entering DNA which it thereby defines as foreign^{46–48}. Restriction cleavage of the DNA will prevent the phage production but not necessarily expression of much of its genome. Subsequently, breakdown prevents, in addition, most gene expression²⁹ but still makes possible rescue of many genetic loci^{23,49}. We are currently investigating the expression of the cleavage products of phage DNA *in vivo*.

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- ¹ *Science*, **187**, 931–935 (1975).
- ² Nathans, D., and Smith, H. O., *A. Rev. Biochem.*, **44**, 273–293 (1975).
- ³ Meselson, M., Yuan, R., and Heywood, J., *A. Rev. Biochem.*, **41**, 447–446 (1972).
- ⁴ Yuan, R., Bickle, T. A., Ebberts, W., and Brack, C., *Nature*, **256**, 556–560 (1975).
- ⁵ Greene, P. J., et al., *J. molec. Biol.*, **99**, 237–261 (1975).
- ⁶ Polisky, B., et al., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 3301–3314 (1975).
- ⁷ Schlagman, S., Hattman, S., May, M. S., and Berger, L., *J. Bact.* (in the press).
- ⁸ Studier, F. W., *J. molec. Biol.*, **94**, 283–295 (1975).
- ⁹ Meselson, M., and Yuan, R., *Nature*, **217**, 1110–1114 (1968).
- ¹⁰ Smith, H. O., and Wilcox, K. W., *J. molec. Biol.*, **51**, 379–391 (1970).
- ¹¹ Horiuchi, K., and Zinder, N. D., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3220–3224 (1972).
- ¹² Murray, N. E., Batten, B. L., and Murray, K., *J. molec. Biol.*, **81**, 395–407 (1973).
- ¹³ Horiuchi, K., Vovis, G. F., and Zinder, N. D., *J. biol. Chem.*, **249**, 543–552 (1974).
- ¹⁴ Horiuchi, K., and Zinder, N. D., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 2555–2558 (1975).
- ¹⁵ Arber, W., *J. molec. Biol.*, **11**, 247–256 (1965).
- ¹⁶ Klein, A., and Sauerbier, W., *Biochem. biophys. Res. Commun.*, **18**, 440–445 (1965).
- ¹⁷ Hattman, S., and Fukasawa, T., *Proc. natn. Acad. Sci. U.S.A.*, **50**, 297–300 (1963).
- ¹⁸ Sheddlovsky, A., and Brenner, S., *Proc. natn. Acad. Sci. U.S.A.*, **50**, 300–306 (1963).
- ¹⁹ Symonds, N., Stacey, K. A., Glover, S. W., Schell, J., and Silver, S., *Biochem. biophys. Res. Commun.*, **12**, 220–222 (1963).
- ²⁰ Hedgpeth, J., Goodman, H. M., and Boyer, H. W., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3448–3452 (1972).
- ²¹ Dussoix, D., and Arber, W., *J. molec. Biol.*, **5**, 37–49 (1962).
- ²² Fukasawa, T., *J. molec. Biol.*, **9**, 525–536 (1964).
- ²³ Hattman, S., *Virology*, **24**, 333–348 (1964).
- ²⁴ Paigen, K., and Weinfield, H., *Virology*, **19**, 565–572 (1963).
- ²⁵ Glover, S. W., *Uptake of Informative Molecules by Living Cells* (edit by Ledou, L.), 185–211 (North-Holland, Amsterdam, 1972).
- ²⁶ Arber, W., *Progress in Nucleic Acid Research and Molecular Biology*, **14** (edit. by Cohn, W. E.), 1–34 (Academic, New York, 1974).
- ²⁷ Simmon, V. F., and Lederberg, S., *J. Bact.*, **112**, 161–169 (1972).
- ²⁸ Barbour, S. D., and Clark, A. J., *Proc. natn. Acad. Sci. U.S.A.*, **65**, 955–961 (1970).
- ²⁹ Silverstein, J., and Goldberg, E. B., *Virology* (in the press).
- ³⁰ Silverstein, J., and Goldberg, E. B., *Virology* (in the press).
- ³¹ Adams, M. H., *Bacteriophages* (Interscience, New York, 1959).
- ³² Shah, D. B., and Berger, H., *J. molec. Biol.*, **57**, 17–34 (1971).
- ³³ Studier, F. W., *J. molec. Biol.*, **11**, 373–390 (1965).
- ³⁴ Malamy, M. H., Fiandt, M., Szybalski, W., *Molec. gen. Genet.*, **117**, 207–222 (1972).
- ³⁵ MacKay, V., and Linn, S., *J. biol. Chem.*, **249**, 4286–4294 (1974).
- ³⁶ Revel, H. R., *Virology*, **31**, 688–701 (1964).
- ³⁷ Fleischman, R. A., and Richardson, C. C., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2527–2531 (1971).
- ³⁸ Hewlett, M. J., and Mathews, C. K., *J. Virol.*, **15**, 776–784 (1975).
- ³⁹ Altman, S., and Meselson, M., *Proc. natn. Acad. Sci. U.S.A.*, **66**, 716–721 (1970).
- ⁴⁰ Tanner, D., and Oishi, M., *Biochim. biophys. Acta*, **228**, 764–769 (1971).
- ⁴¹ Sakaki, Y., *J. Virol.*, **15**, 1611–1612 (1974).
- ⁴² Dharmalingam, K., and Goldberg, E. B., *Nature*, **260**, 000–000 (1976).
- ⁴³ Benzinger, R., and McCorquodale, D. J., *J. Virol.*, **16**, 1–4 (1975).
- ⁴⁴ Studier, F. W., *J. molec. Biol.*, **94**, 283–295 (1975).
- ⁴⁵ Kornberg, A., *DNA Synthesis*, (Freeman, San Francisco, 1974).
- ⁴⁶ Luria, S. E., and Human, M. L., *J. Bact.*, **64**, 557–569 (1952).
- ⁴⁷ Bertani, G., and Weigle, J. J., *J. Bact.*, **65**, 113–121 (1953).
- ⁴⁸ Revel, H. R., and Luria, S. E., *A. Rev. Genet.*, **4**, 177–192 (1970).
- ⁴⁹ Terzi, M., *J. molec. Biol.*, **34**, 165–173 (1968).

letters to nature

Ariel V sky survey observations of X rays from the globular cluster candidates 3U2131+11 and MXO513–40

THE high energy (2–18 keV) detector system of the Ariel V X-ray sky survey experiment (SSE) has observed the faint, high galactic latitude X-ray sources 3U2131+11 and MXO513–40 on a number of separate occasions over a period of a year (January–December 1975) and the results are reported here. These sources have previously been observed by OSO-7 (and

3U2131+11 also by Uhuru), and the globular clusters NGC 7078 and NGC 1851 respectively, were proposed as identifications^{1,2}. Variability of the timescales of days to months was also observed².

The SSE instrument and data analysis are described elsewhere (ref. 3 and G. Villa *et al.*, unpublished). The 90% confidence error box for the source location is determined from combining the individual positional measurements by calculating the product of the probabilities of the source having been found at any given point during a single scan^{4,5}.

Figures 1 and 2 show the error boxes for 3U2131+11 and

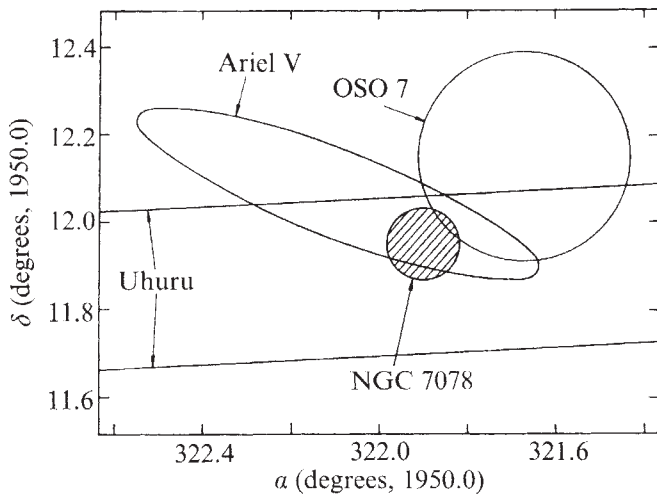


Fig. 1 Positional error boxes for the X-ray source 3U2131+11 associated with NGC 7078, from Ariel V, OSO-7 and Uhuru.

MXO513-40 from the Ariel V sky survey, together with those from OSO-7 (ref. 2, and from Uhuru¹, for 3U2131+11). The Ariel V error boxes reduce the uncertainty in the locations of the sources and support the previous identifications^{1,2} of 3U2131+11 = NGC 7078 and MXO513-40 = NGC 1851. The centroids of our 90% confidence error boxes have celestial coordinates (α , δ , 1950.0) and areas as follows

3U2131+11: (322.10°, 12.07°), 0.15 degree²

MXO513-40: (78.19°, -40.13°), 0.066 degree²

Figures 3 and 4 show, for the two X-ray sources, the intensities observed by Ariel V. Each data point consists of a summation of observations over a period of 1-2 d. Clark *et al.*² have reported variability of factors ~ 2 and ~ 5 for 3U2131+11 and MXO513-40 respectively, on timescales of days to months. For 3U2131+11 the intensities measured by Ariel V (Fig. 3) are consistent with a constant value of ~ 2 Ariel V counts s⁻¹ (2-18 keV). They are also consistent with the variability ~ 2 reported from OSO-7 (ref. 2), but it seems unlikely, from Fig. 2, that the variability greatly exceeds this level, as all 9 statistically significant intensity measurements ($\geq 3\sigma$) from Ariel V are within a factor 2 of each other.

The Ariel V observations of MXO513-40 show intensity variations of a factor ~ 6 over periods of ~ 10 d, consistent with the OSO-7 data². The range of intensities recorded was ~ 1 -7 Ariel V count s⁻¹ (1 Ariel V count $\simeq 2.7$ Uhuru counts, 1 OSO-7 counts $\simeq 15$ Uhuru counts^{6,7} for a spectrum like that of the Crab Nebula). Of particular interest is a set of six obser-

vations centred on 17 February 1975 (MJD42460) covering a period of ~ 20 d (see Fig. 4). These observations suggest flare-like behaviour, the source intensity increasing from < 1 to ~ 6 Ariel V counts s⁻¹ in 12 d and then falling back to the original level on a similar timescale. Combining the two 'flares' with the Ariel V data at $\sim$ MJD42460 and 42605, with a similar emission peak from OSO-7 at $\sim$ MJD41700 (see Fig. 4), reveals a possible periodicity in flaring of (160 ± 20) d (or sub-multiple of this period).

Being highly evolved, globular clusters appear ideal sites for capture binary systems or massive central black holes. Both have been used in explaining the globular cluster X-ray sources, the emission being assumed to result from appreciable matter

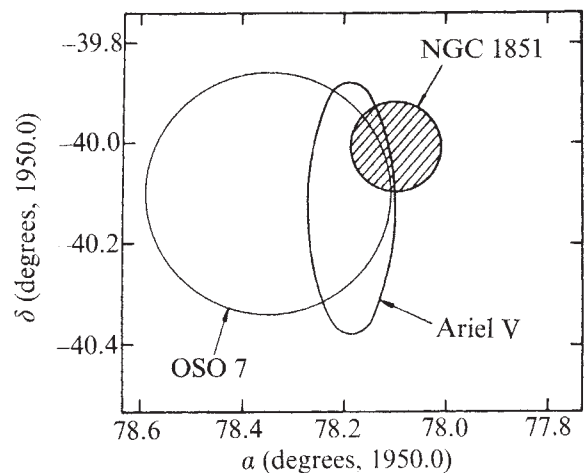


Fig. 2 Positional error boxes for the X-ray source MXO513-40 associated with NGC 1851, from Ariel V and OSO-7.

accretion (see refs 8-11). In each case the X-ray emission should arise close to the centre of the cluster, and our results are consistent with such a location. For MXO513-40, our X-ray error boxes do not favour identification with any particular optical object in NGC 1851, of those that have been suggested^{12,13}, though it may be significant that both the Ariel V and OSO-7 error boxes lie to the same side of the cluster centre. Therefore it is necessary to obtain a still more precise location for the X-ray source.

Finally, we note that none of the theories so far put forward explicitly accounts for the variability on timescales of days and longer, observed by Ariel V and OSO-7. Thus it is important to monitor the X-ray emission and search for correlated variability in the candidate optical counterparts. X-ray spectral data will also be useful in testing the theories for the emission^{6,11}.

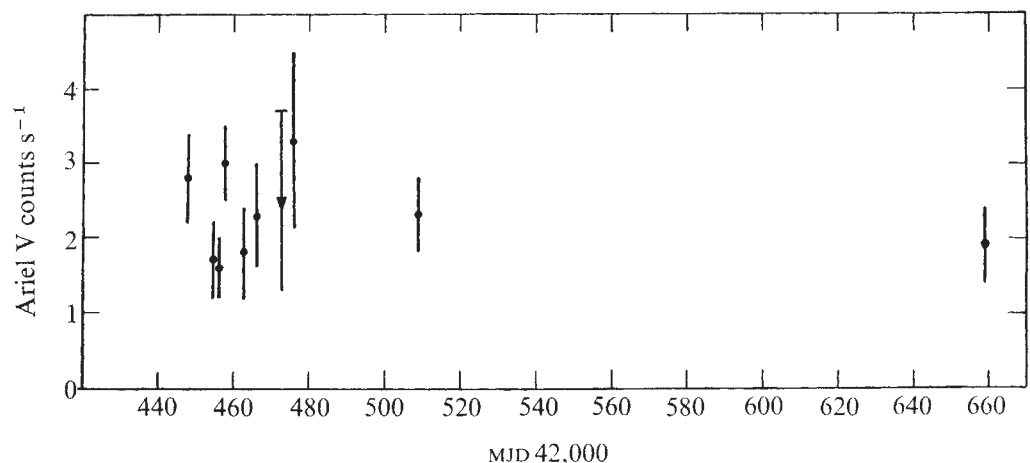


Fig. 3 Ariel V observations of intensity against time for 3U2131+11. Error bars are $\pm 1\sigma$.

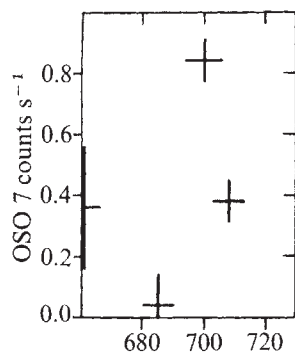
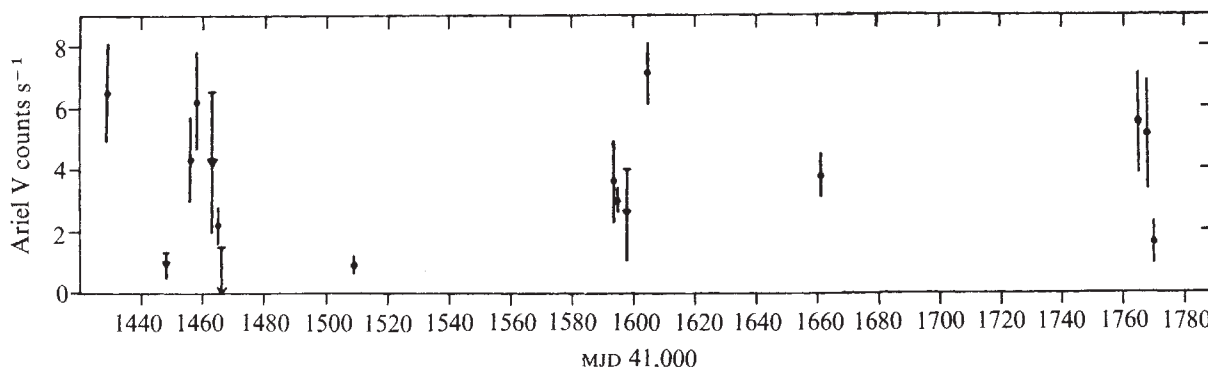


Fig. 4 Observations of intensity against time for MXO513-40: (upper panel) OSO-7 (from ref. 2); (lower panel) Ariel V. Error bars are $\pm 1\sigma$.



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Note added in proof: Jones and Forman have recently reported¹⁴ an Uhuru observation of an X-ray burst from the vicinity of MXO513-40. We note that the peak intensity of MXO513-40 observed by Ariel V could be supplied by about two such bursts per orbit.

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- ¹ Giacconi, R., et al., *Astrophys. J. Suppl.*, **27**, 37 (1974).
- ² Clark, G. W., Markert, T. H., and Li, F.-K., *Astrophys. J. Lett.*, **199**, L97 (1975).
- ³ Ricketts, M. J., Cooke, B. A., and Pounds, K. A., *Nature*, (in the press).
- ⁴ Giacconi, R., and Gursky, H. (eds), *X-ray Astronomy* (Reidel, Holland, 1974).
- ⁵ Davison, P. J. N., *Nature phys. Sci.*, **246**, 90 (1973).
- ⁶ Clark, G. W., *Proc. Enrico Fermi Summer School Phys. Astrophys. of Neutron Stars and Black Holes*, Varenna, Italy (1975).
- ⁷ Winkler, P. F., and White, A. E., *Astrophys. J. Lett.*, **199**, L139 (1975).
- ⁸ Clark, G. W., *Astrophys. J. Lett.*, **199**, L143 (1975).
- ⁹ Fabian, A. C., Pringle, J. E., and Rees, M. J., *Mon. Not. R. astr. Soc.*, **172**, 15P (1975).
- ¹⁰ Silk, J., and Arons, J., *Astrophys. J. Lett.*, **200**, L131 (1975).
- ¹¹ Bahcall, J. N., and Ostriker, J. P., *Nature*, **256**, 23 (1975).
- ¹² Liller, M. H., *Astrophys. J. Lett.*, **201**, L125 (1975).
- ¹³ Vidal, N. V., and Freeman, K. C., *IAU Circ.*, No. 2744 (1975).
- ¹⁴ Jones, C., and Forman, W., *IAU Circ.*, No. 2913 (1976).

New galactic population class

GALACTIC γ rays have been shown to come from regions where H_2 is dense, and where also 'population I' objects are predominantly found. The existence of such regions can be interpreted using the density-wave model of spiral galaxies, and leads us to propose here a change in the Baade classification scheme: 'population I' now to include all the objects associated with the H_2 clouds, and a new class, 'population O', to refer to the regions, relatively richer in atomic hydrogen, which lie further from the galactic centre.

Satellite observations¹ imply that γ -ray emission is highly non-uniform in the Galaxy, and that its emissivity distribution peaks about halfway between the Sun and the galactic centre²⁻⁴. Analysis of the final SAS-2 data places this peak emissivity in the region between 5 and 6 kpc from the galactic centre⁵. The γ -ray emissivity distribution bears a strong similarity to

the distribution of molecular clouds in the Galaxy⁶ which also peaks in the 5-6-kpc region^{7,8}. This similarity, coupled with the lack of enough gas in atomic form (H I) to explain the γ -ray measurements led to the supposition that H_2 is far more abundant in the inner Galaxy than H I, and that H_2 has the major role in producing galactic γ rays^{6,8,9}. In fact a γ -ray emissivity which scales like the more uniform H I distribution cannot explain the observations. Recent studies indicate that the volume averaged density of H_2 is $\sim 2-3$ molecules cm^{-3} in the 5-6-kpc region and drops off dramatically at < 4 kpc and in the outer Galaxy, so that at 10 kpc at least half of the interstellar gas is probably in atomic form⁹. There is a negligible amount of H_2 in the outer regions of the Galaxy^{7,8}. A deduction of the implied cosmic-ray distribution from the γ -ray observations shows that the cosmic rays increase (relative to the local intensity) by a factor of ~ 2 (ref. 9) or slightly more⁵ at a maximum coincident with the maximum in the gas density, in the 5-6-kpc region, and that the cosmic rays drop off rather rapidly in the outer Galaxy^{9,10}. The cosmic-ray distribution deduced using the γ -ray observations in conjunction with the deduced variation of total gas ($H I + H_2$) in the Galaxy is, within experimental error, identical to the distribution of supernova remnants¹¹ and pulsars (refs 12 and 13, and J. Sieradakis, personal communication). The similarity of the deduced cosmic-ray distribution and the distribution of supernova remnants provides our strongest evidence to date that the observed cosmic-ray nucleons, which make up 99% of cosmic rays, originate in galactic supernovae, in either the explosion or the resulting pulsars¹⁴.

On the large scale, therefore, there appears to be an excellent correlation between several important constituents of the Galaxy in terms of their distributions as a function of galactocentric distance. These constituents are molecular clouds, H II regions (ionised hydrogen), cosmic rays, γ rays, supernova remnants and pulsars. All of these constituents of the Galaxy seem to be densest in the 5-6-kpc region and seem to drop off sharply inside of 4 kpc and in the outer Galaxy. They all can be associated with the formation and evolution of the population I stars in the Galaxy and are known to have a population I distribution^{15,16}. They are associated with the formation and destruction of hot young O and B stars in the Galaxy, which delineate the arms in other spiral galaxies. That the correlation of these components is natural can best be seen in Fig. 1. The gravitational collapse of molecular clouds is expected to lead

to the formation of OB associations containing the massive, hot, short lived O and B stars whose ultraviolet radiation causes the formation of zones of ionised gas around them (H II regions). The massive O and B stars, after a few million years, terminate their existence as supernovae, which in turn leads to the generation of cosmic rays. It has also been suggested that supernova explosions can trigger the formation of new OB associations in a feedback effect (ref. 17, and H. B. Ogelman and S. P. Maran, unpublished). The compound effect of cosmic rays and molecular clouds being enhanced in the same region of the Galaxy then leads to an even stronger enhancement of the γ -ray emissivity in this region. In addition, an increase in the flux of subrelativistic cosmic rays may help lead to an increase in the amount of ionised gas in the region ~ 5 kpc, as indicated in recent surveys (ref. 18 and F. J. Lockman, personal communication).

Whereas all of the above components of the galaxy have correlated large scale galactic distributions, 21-cm radio observations of H I indicate a relatively constant overall density distribution of atomic hydrogen between 4 and 14 kpc from the galactic centre with no evidence for a significant enhancement in the 5–6-kpc region^{8,19}. This implies that the H_2 distribution is much more sensitive to the compression effects expected in density-wave models of galactic structure than the more diffuse H I with the ratio $H_2/H I$ having a radial galactic dependence somewhat similar to that of H II/H I as discussed by Shu²⁰.

There is a large variation in structural details among spiral galaxies. This range of detail, from those with long thin well developed arms and high surface brightness (van den Bergh type I) to those with only a bare hint of arm structure (van den Bergh type V) has been incorporated into the general framework of density wave theory by Roberts *et al.*²¹. The galaxies with well developed arms and high surface brightness with an implied star formation rate are found to satisfy the condition $(W_1/a) > 1$ where W_1 is the velocity component of basic rotation normal to the spiral arms and a is the effective acoustic speed of the interstellar gas. In the inner regions of galaxies, there can exist zones of strong nonlinear compression where $(W_1/a) > 1$ and in the outer regions, zones of weak linear compression where $(W_1/a) < 1$. Burton²² has estimated that the interface between these two zones in our Galaxy occurs at a galactocentric radius $R \sim 10$ kpc.

Figure 2 shows the smoothed radial distribution of mean surface densities of the atomic and molecular components of interstellar gas in our Galaxy based on the recent data of Burton *et al.*⁹, where the H_2 density is normalised according to the methods of Stecker *et al.*⁹ and using a scale height of ~ 65 pc for the molecular cloud distribution (based on the larger sample given in ref. 7). Also shown are the regions of weak and strong compression. It can be seen that the transition region near 10 kpc is one in which the total surface density is roughly constant but where larger and larger amounts of gas are

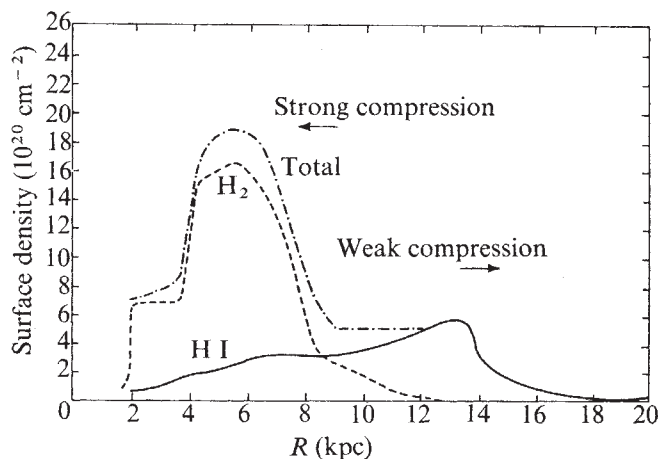


Fig. 2 Surface density distribution of H I, H_2 and total gas as a function of galactocentric radius (see refs 8, 9). The H_2 data are slightly different than those given in ref. 7, but can be considered qualitatively the same for the purpose of the present discussion. The graph illustrates the general separation of H I and H_2 components in the Galaxy and the correlation of these components with the weak compression and strong compression regions of the galactic disk respectively.

converted from H I to H_2 as R decreases.

These recent observational and theoretical developments (and see also ref. 22) prompt me to suggest the following changes in the Baade²³ classification scheme for galactic objects: first, the classification 'population II', which consists of old disk stars ('high velocity stars'), nuclear bulge stars, halo stars and globular cluster stars stays the same.

Second, the classification 'population I' should be expanded to include all galactic objects narrowly confined in the galactic plane and associated with the formation of population I stars. Thus the set of galactic objects of population I will include molecular clouds, OB associations, H II regions, dark nebulae, dust, supernovae, and even associated radiation fields such as infrared, synchrotron and π^0 -decay γ radiation from molecular clouds. This population is expected to predominate in regions of the galaxy where $(W_1/a) > 1$ (strong compression)^{8, 21, 22}.

Third, I define a new class, 'population 0', consisting of the more diffuse atomic hydrogen which is now considered not to have a primary role in star formation. (In the case of some of the denser H I clouds there may be some blurring of definition.) This population will be important in regions where $(W_1/a) < 1$ (weak compression). The main distinction between populations 0 and I stems from the effect of compression with the higher compression regions caused by the nonlinear density waves. These effects result in the marked differences in distribution and dynamics between the two populations. For instance, the population 0 component will have a scale height perpendicular to the galactic plane ≥ 110 pc, and a galactocentric radius of maximum surface density of 12–13 kpc. For population I objects these figures would be ~ 40 –70 pc and 5–6 kpc respectively.

It is found that in late-type spiral galaxies it is characteristic for the neutral hydrogen density to peak well outside the visible radius of the galaxy²⁴. The above classification, with population 0 removed from a primary role in the star formation process, naturally accommodates this hitherto somewhat mysterious fact.

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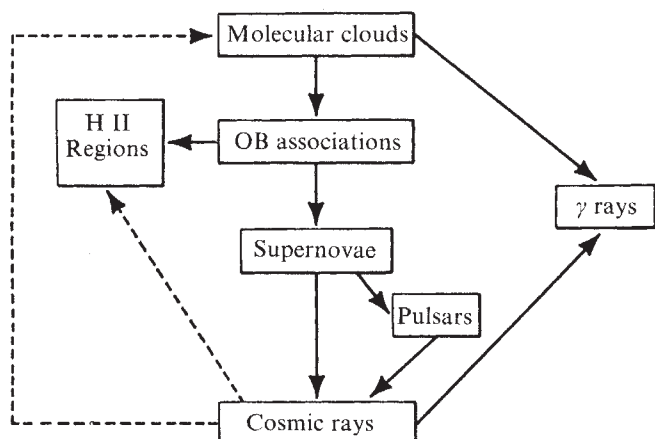
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¹ Fichtel, C. E., *et al.*, *Astrophys. J.*, **198**, 163 (1975).

² Stecker, F. W., Puget, J. L., Strong, A. W., and Bredekamp, J. H., *Astrophys. J. Lett.*, **188**, L59 (1974).

Fig. 1 Relationship between various 'population I' galactic components.



- ³ Puget, J. L., and Stecker, F. W., *Astrophys. J.*, **191**, 323 (1974).
- ⁴ Strong, A. W., *J. Phys. A.*, **8**, 617 (1975).
- ⁵ Puget, J. L., Ryter, C., Serra, A., and Bignami, G. F., *Astr. Astrophys.* (in the press).
- ⁶ Solomon, P. M., and Stecker, F. W., *Proc. ESLAB γ -Ray Symp., Frascati, Italy, ESRO-SP-106*, 253 (1974).
- ⁷ Scoville, N. Z., and Solomon, P. M., *Astrophys. J. Lett.*, **187**, 167 (1975).
- ⁸ Burton, W. B., Gordon, M. A., Bania, T. M., and Lockman, F. J., *Astrophys. J.*, **202**, 30 (1975).
- ⁹ Stecker, F. W., Solomon, P. M., Scoville, N. Z., and Ryter, C. E., *Astrophys. J.*, **201**, 90 (1975).
- ¹⁰ Dodds, D., Strong, A. W., and Wolfendale, A. W., *Mon. Not. R. astr. Soc.*, **171**, 569 (1975).
- ¹¹ Kodaira K., *Publ. astr. Soc. Japan*, **26**, 255 (1974).
- ¹² Lyne, A. G., in *Galactic Radio Astronomy* (edit. by Kerr, F. J., and Simonson, S. C., III).
- ¹³ Hulse, R. A., and Taylor, H. J., *Astrophys. J. Lett.*, **201**, (1975).
- ¹⁴ Stecker, F. W., *Phys. Rev. Lett.*, **35**, 188 (1975).
- ¹⁵ Ilovaisky, A., and Lequeux, J., *Astr. Astrophys.*, **20**, 347 (1972).
- ¹⁶ Gordon, M. A., Brown, R. L., and Gottesman, S. T., *Astrophys. J.*, **178**, 119 (1972).
- ¹⁷ Opik, E., *Irish Astr. J.*, **2**, 219 (1953).
- ¹⁸ Mezger, P. A., *Proc. IAU Symp. No. 38, Switzerland* (edit. by Becker, W., and Contopoulos, G.), 107 (Reidel, Dordrecht, Holland, 1970).
- ¹⁹ Kerr, F. J., and Westerhout, G., in *Galactic Structure Stars and Stellar Systems*, 5 (edit. by Blaauw, A., and Schmidt, M.), 167 (University of Chicago Press, 1965).
- ²⁰ Shu, F. J., in *The Interstellar Medium* (edit. by Pinkau, K.), 219 (Reidel, Dordrecht, Holland, 1973).
- ²¹ Roberts, W. W., Jr., Roberts, M. S., and Shu, F. H., *Astrophys. J.*, **196**, 381 (1975).
- ²² Burton, W. B., *Jr. Astr. Astrophys.* (in the press).
- ²³ Baade, W., *Astrophys. J.*, **100**, 137 (1944).
- ²⁴ Roberts, M. S., *Science*, **183**, 371 (1974).

Volcanic triggering of glaciation

AN instantaneous glaciation model for the formation of the large Pleistocene ice sheets has been proposed by Flohn¹ and by Ives *et al.*². It depends on the sudden buildup of a permanent snow cover over sub-Arctic upland plateaux including Baffin Island, Ungava, Labrador and Keewatin, Melville Peninsula, and the Finnmark Plateau and upland Sweden. The crucial event in this sudden snow buildup is the survival of snow over a large area for a single summer which then results in a series of feedback reactions leading to the establishment of permanent snowfields and, subsequently, icefields. I suggest here that such a survival could have resulted from one or several closely spaced massive volcanic ash eruptions.

Barry *et al.*³ have stated that the climatic change needed to initiate the buildup of large permanent sub-Arctic snowfields may be quite small, but no quantitative estimates have been made of the conditions which could result in the large-scale survival of snow for a single summer. The most likely of these conditions would be a greater than normal winter snow accumulation followed by a cold, cloudy (or hazy), snowy summer, with a dearth of warm storms and a greatly decreased input of solar radiation. Volcanic eruptions over the past several centuries for which climatic data are available have enhanced these conditions, as is evidenced first, by a decrease in solar radiation which in latitudes 30–50° varies from 20 to 30% (refs 4 and 5); second, a decrease in the daylength of effective summer insolation⁶; third, a decline in mid-latitude yearly temperatures of 0.5–1.8 °C and in summer temperatures of up to 2–3 °C, (these declines lasting from 3 to 7 yr, and 10–15 yr at the higher latitudes)⁶⁻⁷; and fourth, a possible increase in precipitation (many of the coldest and wettest summers in Europe, North America and Japan have followed volcanic eruptions⁸⁻⁹) resulting from increased stratospheric dust of micron and sub-micron size which could descend into the troposphere and serve as freezing nuclei⁹.

The effects of volcanism on temperature would be at least twice as great at the higher latitudes judging by temperature data supplied by Schell¹⁰. Volcanism would also lead to fewer warm storms over the sub-Arctic, which would mean less snow pack ablation^{7,11}, and to a higher frequency and intensity of summer snow storms with a resulting increase in albedo.

Such climatic effects came to maxima during the two largest volcanic eruptions since AD 1500—in 1815 and 1835. The eruptions respectively had Dust Veil Indexes of 3,000 and 4,000, total volume of tephra of 100–300 km³ and 50–150 km³ and volumes of atmospheric dust production of around 15 km³ and 12 km³ (ref 6). These eruptions were considerably less intense than some of the large Pleistocene eruptions, one of

which¹² had a rhyolitic pumice and ash production of more than 900 km³. The maximum yearly mid-latitude temperature decline in the first several years following the 1815 and 1835 eruptions was around 1.0 to 1.7 °C, and the maximum decline in summer temperatures was 2.2–2.3 °C. The temperature depression following a massive eruption of around 900 km³ would depend on the total atmospheric ash production from the eruption and could be at least three times those averages in mid-latitudes and at least six times in sub-Arctic regions. There is also the possibility that a massive eruption north of the equatorial region at the right time of year would block almost all direct solar radiation during the entire summer in the sub-Arctic.

Considering the possible climatic effects of volcanism, I suggest that a massive tephra eruption (that is, 300–900 km³ or larger) could have produced sufficient atmospheric dust to promote the summer survival of the snow pack on sub-Arctic plateaux. Survival would be especially likely if there were large eruptions two or more years running. Once such a survival of summer snow had occurred, it could have led to the autocatalytic growth of permanent snowfields as outlined by Flohn¹ with the following modifications: first, as a result of the original ash eruption or eruptions, the feedback process would be reinforced, leading to a greater probability of snowfield survival in the second and subsequent summers, since the lowered temperatures and decreased solar radiation would occur for at least several summers, especially at the higher latitudes; second, increased albedo and lower temperatures could result from an increase in the area of sea ice (such as occurred after the 1912 Katmai eruption¹³), which would augment the increased land albedo resulting from the survival of summer snow. Flohn¹ has indicated that the high surface albedo of a snowfield that has survived a single summer would prevent its early destruction and has outlined some steps by which a 15-m tall forest on the Ungava plateau could be covered by snow within 22 yr after a summer in which the upland snow did not melt.

If a sub-Arctic snow pack survived into early or mid-summer as a result of a massive ash eruption, then there is a possibility that the thermal condition of the land surface could contribute to further snow survival. An example¹⁴ of this sort of feedback mechanism is the anomalous summer of 1968 during which cold sodden ground and an increased level of snow survival in parts of northern Eurasia in the early summer led to the formation of a stationary upper cold trough over north-eastern Europe and north-western Siberia. The trough persisted with the aid of thicker sea ice to the north, and it contributed to the survival of the snow pack in some areas until late June or early July and to the occurrence of early snowfalls from late July to late August. Flohn¹ has suggested that such a superimposed cold low resulting from the large-scale survival of summer snow would force the upper air to curve cyclonically and thus increase snowfall.

There is evidence of an increased area of permanent snow on the sub-Arctic plateau during the recent Little Ice Age^{2,3,14,15} and during a previous neoglaciation phase around 2,400 yr BP (ref. 16). It is not possible to estimate the relative importance of volcanism in those increases, though both periods were characterised by significant increase of volcanic activity¹⁷. Whatever the total increased area of snowfield, it was not sufficient to trigger the formation of a large ice sheet, and as a consequence both periods could be considered "abortive glaciations" in the sense of Flohn¹ and Ives *et al.*². Had a massive eruption occurred during these phases of increased volcanism and glaciation, then it is quite conceivable that an increase in the area of permanent snow cover might have occurred to the point at which the albedo increased and temperature fell sufficiently to permit the snowfields to survive. If that occurred, then large, mid-latitude ice sheets may have subsequently formed.

For the volcanic triggering proposed here to operate, the Earth must be in the proper stage of its glacial-interglacial cycle, that is, a genuine interglacial must be occurring so that there is sufficient heat in the oceans to energise the transport of large volumes of water over the sub-polar plateaux¹⁸.

If a massive ash eruption occurs when the Earth is experiencing a major ice-sheet advance, then it would have little climatic impact, nor, according to Adam's model¹⁸, would a large eruption have much impact in the early stages of glacial retreat. If, however, a large ash eruption occurred after a massive retreat of the mid-latitude ice sheets, then sufficient cooling over upland plateaux could result in year-round snow cover. If so, then a major glacial readvance could occur, always assuming that sufficient heat had been stored in the oceans to transfer enough water to the snowfields.

The possibility that large Pleistocene ice sheets were initially triggered by massive volcanic eruptions is not contradicted by the geological record. There is an apparent correlation between major phases of global ice advance and ash eruptions in New Zealand, Japan and southern South America over the past 42,000 yr (ref. 17). A compilation of further information on volcanism from these areas and from North America and Europe, which I have recently made, supports this conclusion. The much higher rate of explosive volcanism throughout the Pleistocene has already been suggested as an indication of a possible link between volcanism and glaciation^{19,20}.

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Received November 11, 1975; accepted February 8, 1976.

- ¹ Flohn, H., *Quat. Res.*, **4**, 385–404 (1974).
- ² Ives, J. D., Andrews, J. T., and Barry, R. G., *Naturwissenschaften*, **62**, 118–125 (1974).
- ³ Barry, R. G., Andrews, J. T., and Mahaffy, M. A., *Science*, **190**, 979–981 (1975).
- ⁴ Dyer, A. J., and Hicks, B. B., *Nature*, **208**, 131–133 (1965).
- ⁵ Lamb, H. H., *Palaogeogr. Paleoclimatol. Palaeoecol.*, **10**, 203–230 (1971).
- ⁶ Lamb, H. H., *Proc. R. Soc. A*, **266**, 425–533 (1970).
- ⁷ Lamb, H. H., *Adv. ecol. Res.*, **7**, 177–233 (1971).
- ⁸ Arakawa, H., *Pap. Meteorol. Geophys.*, **6**, 101–115 (1955).
- ⁹ Rosinski, J., and Pierrard, J. M., *J. atmos. terr. Physics*, **24**, 1017–1030 (1962).
- ¹⁰ Schell, I. I., *Tufts meteorol. Stud.*, **58**, 1 (1958).
- ¹¹ Bray, J. R., *Nature*, **248**, 42–43 (1974).
- ¹² Eaton, G. R., et al., *Science*, **188**, 787–796 (1975).
- ¹³ Lamb, H. H., and Johnson, A. I., *Geogr. Annlr.*, **41**, 94–134 (1959).
- ¹⁴ Lamb, H. H., *Climate: Present, Past and Future* (Methuen, London, 1972).
- ¹⁵ Andrews, J. T., Davis, P. T., and Wright, C., *Ice*, **47**, 5 (1975).
- ¹⁶ Bassett, I. R., and Terasmae, J., *Can. J. Bot.*, **40**, 141–150 (1962).
- ¹⁷ Bray, J. R., *Nature*, **252**, 679–680 (1974).
- ¹⁸ Adam, D. P., *Interim. res. Rep.*, No. 15, Department of Geochronology, University of Arizona, Tucson (1969); *I.N.Q.U.A. Abs.*, **1**, (INQUA Christchurch, New Zealand, December, 1973).
- ¹⁹ Kennett, J. P., and Thunell, R. C., *Science*, **187**, 497–503 (1975).
- ²⁰ Stewart, R. J., *Nature*, **258**, 505–506 (1975).

Changes in V_P/V_S before the Tokachi-oki earthquake of May 16, 1968 off NE Japan

THE detection of premonitory variations in the ratio of compressional to shear wave velocities is one of the most promising tools for earthquake prediction^{1,2}. Changes in V_P/V_S for some moderate sized earthquakes ($M \approx 5$ –6) located on Japanese islands have been found^{3,4}, and for shallow, small to moderate sized earthquakes ($M < 7$) located on continents. For large submarine earthquakes ($M \approx 8$), however, velocity changes have not previously been reported.

The Tokachi-oki earthquake of May 16, 1968 ($M = 7.9$; focal depth 0 km) occurred near the junction of the Japan Trench and Kurile-Kamchatka Trench. Although earlier work³ had shown no changes in seismic velocities (nor for the Sanriku-oki earthquake ($M = 7.5$) of 1960, also in the same area), I have carefully reanalysed arrival times before these two earthquakes and found significant variations in V_P/V_S . So far as I know, this is the first time that a change in V_P/V_S before and after a great submarine earthquake ($M \approx 8$) has been detected.

The events that occurred in the focal zone of the Tokachi-oki earthquake (May 16, 1968) during 1951–74 were selected from

the Seismological Bulletin of the Japan Meteorological Agency for analysis. The 1968 Tokachi-oki epicentre and after-shock area, together with the stations used for this analysis are shown in Fig. 1. The swarm earthquakes of 1952 (maximum $M = 6.5$) and the Sanriku-oki earthquake of 1960 are also included. Three station combinations, such as Hachinohe(HAC)–Aomori(AOM), Miyako(MIY)–Akita(AKI) and Urakawa(URA)–Obihiro(OBI) were selected for the V_P/V_S calculations. The apparent ratio of compressional to shear velocities was calculated by

$$V_P/V_S = [t_s(A) - t_s(B)] / [t_p(A) - t_p(B)]$$

where t_p and t_s denote arrival time of P and S waves at the corresponding stations. The V_P/V_S ratio from each combination was selected with the restrictions that the source depth must be ≤ 40 km, and that $1.00 < V_P/V_S \leq 2.00$.

The results are illustrated in Fig. 2. Figure 3 shows a typical example of Δt_s against Δt_p for chosen periods of time for the HAC–AOM combination. Long range premonitory changes in V_P/V_S before the 1968 Tokachi-oki earthquake are clearly seen for the HAC–AOM and URA–OBI combinations, but less so for the MIY–AKI combination. These anomalies in

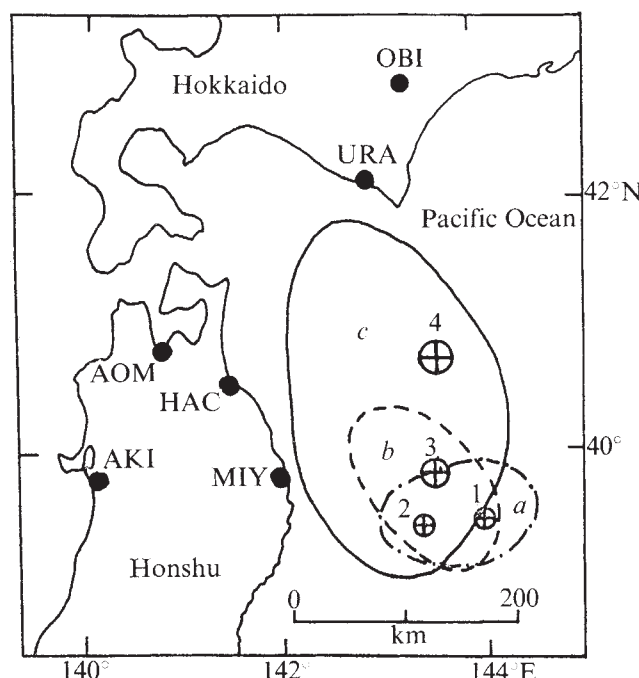


Fig. 1 Locations of the 1968 Tokachi-oki earthquake and seismograph stations of the Japan Meteorological Agency (●). Region a; area of the swarm earthquakes in October 1952 with the two largest events of magnitude 6.5 (refs 1, 2). Regions b and c; after-shock areas of the Sanriku-oki earthquake of March 21, 1960, with magnitude 7.5 (ref. 3), and the Tokachi-oki earthquake of May 16, 1968, with magnitude 7.9 (ref. 4), respectively.

V_P/V_S cannot be attributed to a biased distribution of events used for the analysis because there is no obvious difference in the geographical distribution of epicentres for the normal period and the anomalous one (see Fig. 4).

This long range variation was disturbed by the 1952 swarms and the 1960 Sanriku-oki earthquake. The change before the latter was especially pronounced. The 1968 earthquake occurred 4 yr after V_P/V_S returned to normal.

We may identify only the latter half of the precursory interval, the so-called recovery phase, as is seen in Fig. 2. Unfortunately, we cannot know the V_P/V_S ratios in the decreasing stage, because of the scarcity of data published by

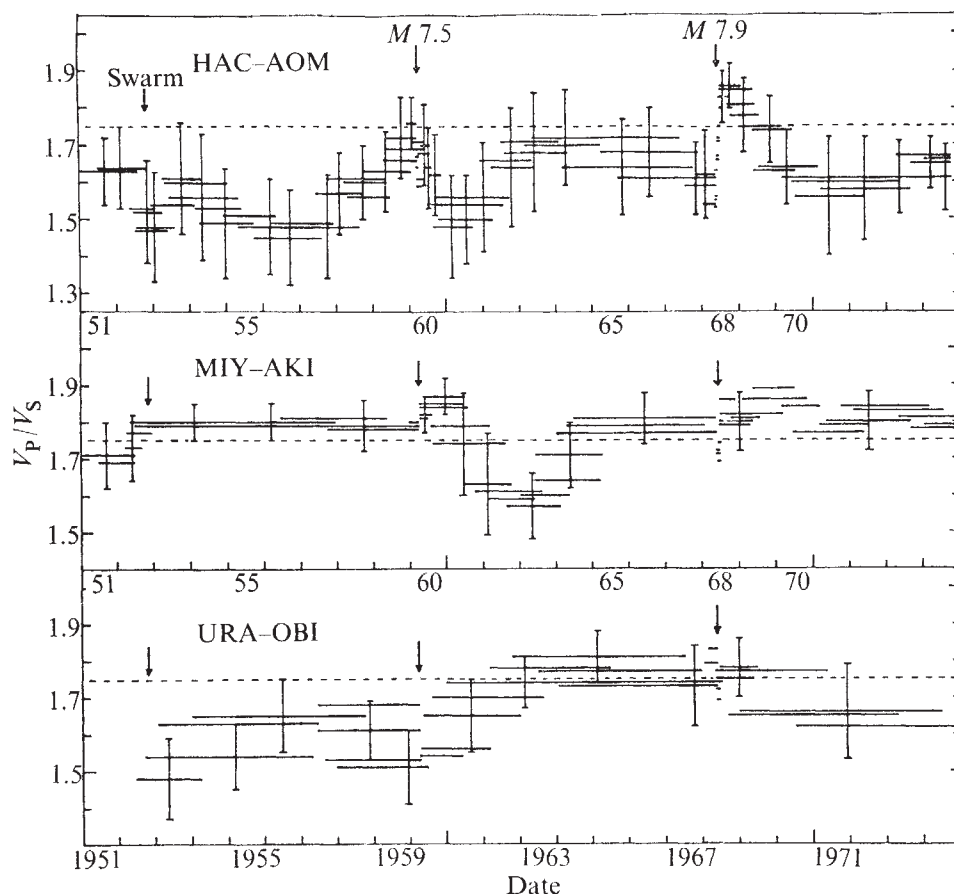


Fig. 2 The variation of V_p/V_s as a function of time before and after the Tokachi-oki earthquake of 1968 for the three station combinations. Horizontal bars show the means for a running time window of ten events stepped by two with its length indicating the time period covered by each window. Vertical bars indicate the 90% confidence limits of the means of some representative windows.

the Japan Meteorological Agency before 1950. The latter half of the precursory interval before the 1968 Tokachi-oki earthquake, however, indicates that a 10–17% decrease in V_p/V_s had occurred, assuming a normal value of 1.75.

Whitcomb *et al.*⁵ have proposed that the earthquake magnitude (M) and precursory anomaly time (t), deduced from V_p/V_s data, are related by

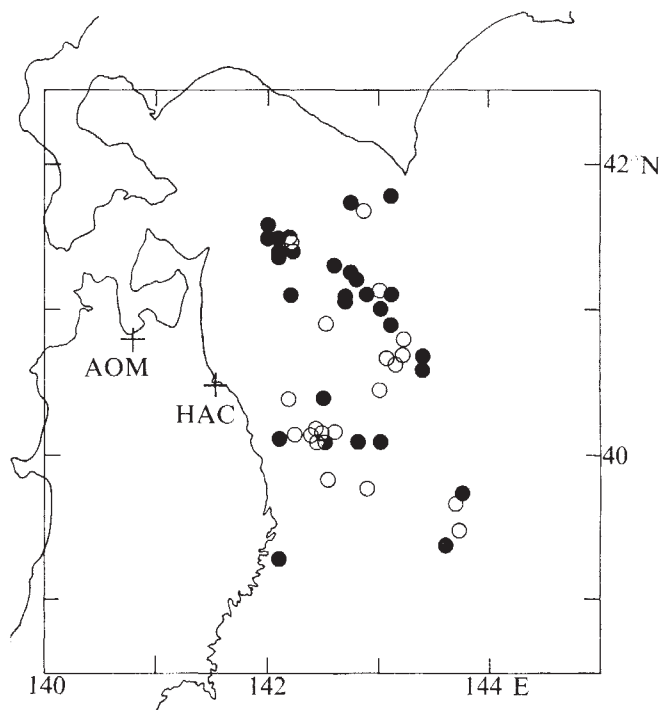
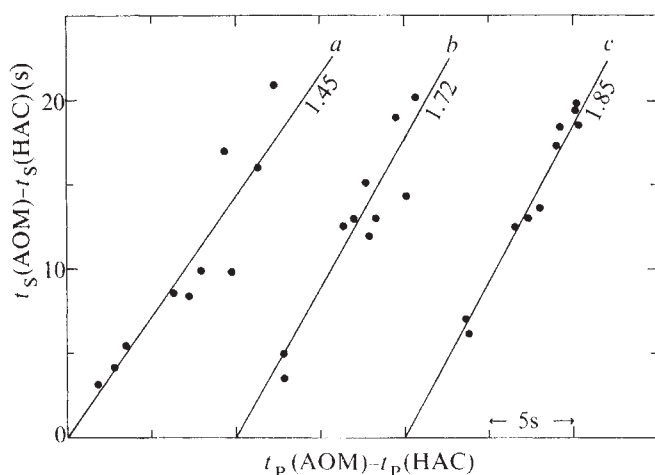
$$\log t = 0.68M - 1.31$$

where t is in days. From this, the precursory intervals are

expected to be 17 yr for an $M = 7.5$ event and 32 yr for $M = 7.9$, respectively. Consequently this analysis is considered to cover almost the whole recovery period, although we cannot decide

Fig. 4 Distribution of epicentres during two typical periods in HAC-AOM combination. ●, Epicentres in the anomalous stage (1953–57); ○, the normal stage (1963–67). There is no obvious difference in geographical distribution of epicentres between these two stages.

Fig. 3 Plots of Δt_s as against Δt_p for three typical periods of time in HAC-AOM combination, each interval including ten events. *a*, Anomalous low stage, October 1, 1955–August 22, 1957, mean and 90% confidence limits $V_p/V_s = 1.45 \pm 0.13$. *b*, Normal stage, May 17, 1963–March 31, 1965, $V_p/V_s = 1.72 \pm 0.13$. *c*, Anomalous high stage, June 26, 1968–May 3, 1969; $V_p/V_s = 1.85 \pm 0.06$.



the starting time of premonitory changes in V_p/V_s ratio for lack of the data.

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Received October 23, 1975; accepted February 11, 1976.

¹ Kisslinger, C., and Wyss, M., *Rev. Geophys. Space Phys.*, **13**, 298-300 (1975).
² Healy, J. H., *Rev. Geophys. Space Phys.*, **13**, 361-364 (1975).
³ Ohtake, M., *J. Phys. Earth*, **21**, 173-184 (1973).
⁴ Hasegawa, A., Hasegawa, T., and Hori, S., *J. Phys. Earth*, **23**, 189-203 (1975).
⁵ Whitcomb, J. H., Garmany, J. D., and Anderson, D. L., *Science*, **180**, 632-635 (1973).

Experimental examination of the gravitational inverse square law

IN a previous paper¹ we pointed out that earlier data on the measurement of the gravitational constant suggest a systematic shift in the value with the separation of the masses. This laboratory has been examining this question for about a year, and the early data gave a discrepancy of 0.4%. New, intensive experiments have given 92 data points, with a resulting discrepancy of 0.37%.

Our experiment compares the gravitational constant at a mass separation of 29.9 cm with the value at a separation of 4.48 cm. To expedite this comparison the gravitational torque signals are kept about the same by using a much larger mass at the greater distance. Following the ingenious suggestion of Faller and Koldweyn (unpublished; see also ref. 2), we have used rings for the attracting mass because the field is very flat at the 'Helmholtz' point and thus the positional accuracy need not be very great. The far ring is made of centrifugally cast brass and has a mass of 57,580.83 g. The near ring is made of pure tantalum with a mass of 1,225.271 g. The far ring has an outside radius of 27.112 cm, an inside radius of 21.589 cm, a thickness

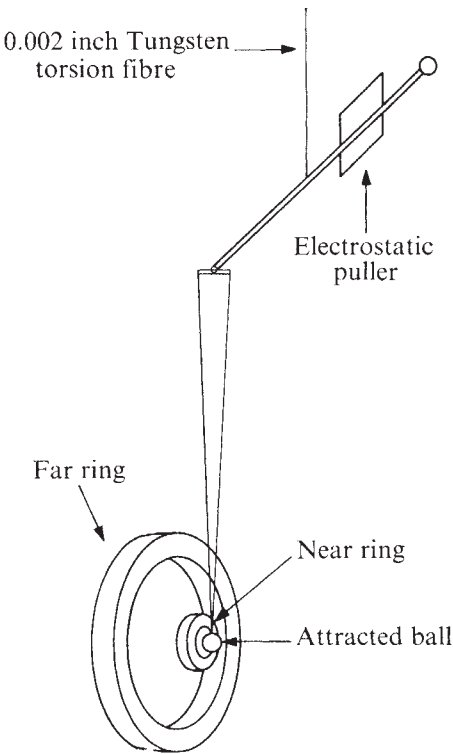


Fig. 1 Apparatus schematic illustrating the positions of the rings. The rings are used one at a time and alternately.

suggested by Liebes. The position of the pendulum was detected by a beam of light which was split into a two-photodiode null detector.

Another important aspect of the experiment was the use of an electrostatic puller to balance the torque of the rings. The experiment was operated in such a way that the attracted ball did not change its position appreciably. The balance voltage (squared) was the measured gravitational torque signal in the experiment; several voltages would be tried which gave an output just slightly off null and the correct null voltage would be interpolated from them.

The quantity measured in the experiment was

Δ = (τ_{far} - τ_{near}) / τ_{near} (1)

where τ_{far} is the total torque produced by the far ring and τ_{near} is the total torque produced by the near ring. The Newtonian value of Δ(Δ_{thy}), was calculated by finding the torque produced by each ring on the attracted ball and each element of the pendulum assembly. Numerical integration over the rings was used to find

Δ_{thy} = 0.03807 ± 0.0005

where the error analysis is given in Table 1.

of 7.633 cm, and the field maximum is located on the ring axis 17.415 cm from the face of the ring. The near ring has an outside radius of 4.5536 cm, an inside radius of 2.7513 cm, a thickness of 1.7765 cm, and the field maximum is located on the ring axis 2.6088 cm from the face of the ring.

The gravitational force was detected with a 48.6-cm Cavendish torsion balance suspended by a 0.002-inch tungsten fibre. The attracted ball, with a mass of 50 g, was made of tantalum and hung by two fibres 87 cm below the balance rod. This reduced the gravitational torques caused by the pendulum rod and counter balance ball. The pendulum hung in a vacuum of 1 × 10⁻⁷ mmHg and the vacuum chamber wall temperature was controlled to ±0.01 °C. Substantial effort went into mounting the apparatus to reduce vibrations (diffusion pumps vibrate badly) and the pendulum hung from a vibration damper

Table 1 Error contributions to Δ_{thy}

Source	Error
Torsion pendulum assembly balance	0.00007
Big ring floor tilt	0.00005
Big ring-pendulum assembly interaction	0.00036
Small ring-pendulum assembly interaction	0.00004
Big ring mass, dimensions and position	0.00031
Small ring mass, dimensions and position	0.00012
Attracted ball sphericity	0.00001
Ring density inhomogeneity	0.0001
Total error	0.00051

Table 2 Error contributions to Δ_{exp}

Standard deviation of the mean of the data	0.00015
Signal against voltage proportionality constant	0.00015
Contact potential	0.00028
Small ring torque	0.00027
Total error	0.00044

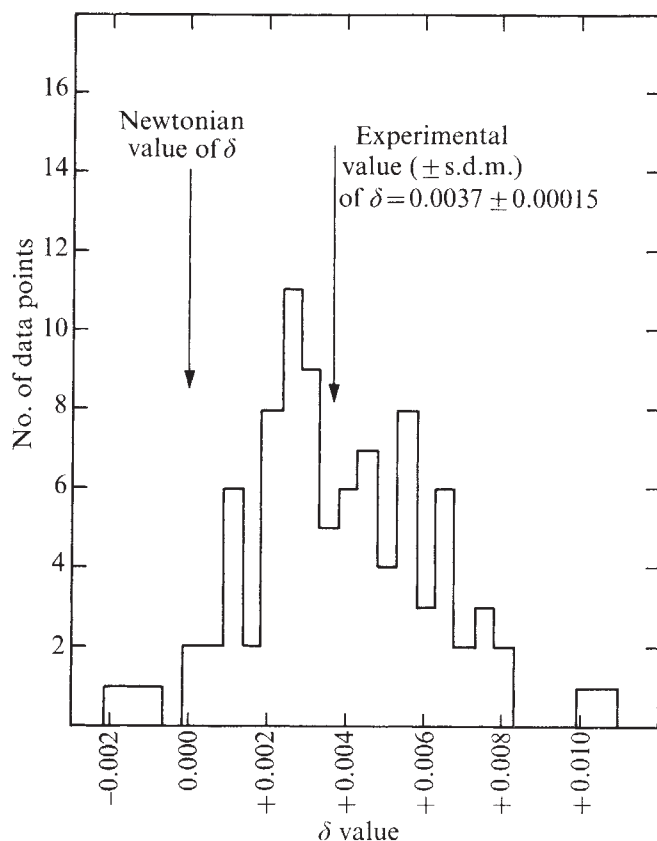


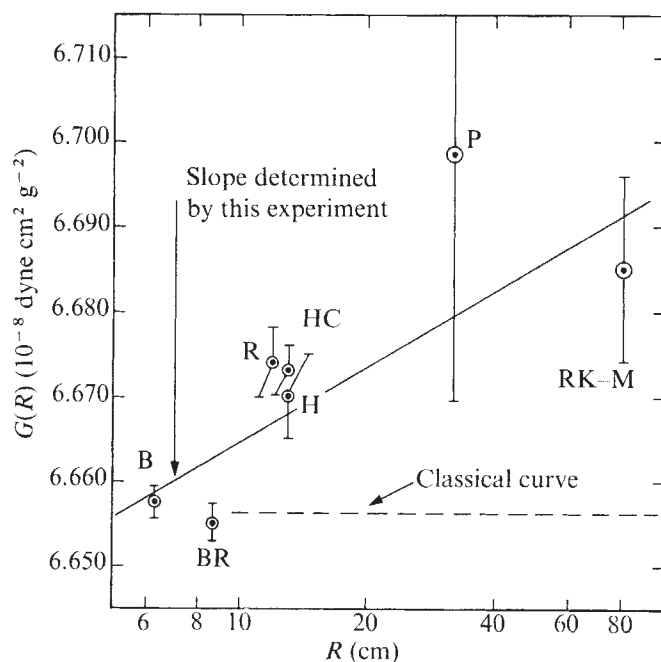
Fig. 2 Histogram of 92 δ values.

Δ_{exp} was found by directly measuring the torque difference $\tau_{\text{far}} - \tau_{\text{near}}$ by first putting one ring and then the other in position. This gave

$$\Delta_{\text{exp}} = 0.04174 \pm 0.0004$$

The discrepancy between the two results is

Fig. 3 The slope of $G(R)$ given by this experiment compared with earlier data. B, ref. 3; BR, ref. 4; R, ref. 5; H, ref. 6; HC, ref. 7; P, ref. 8; RK-M, ref. 9.



$$\delta = \Delta_{\text{exp}} - \Delta_{\text{thy}} = 0.0037 \pm 0.0007$$

The experimental values of δ are given in a histogram in Fig. 2.

A careful re-examination of the experiment indicates that there is no error present large enough to account for this value of δ . In particular, reversal of the rings showed, as theoretically expected, that there is no density inhomogeneity of the rings sufficient to explain this result.

The slope of $G(R)$ from this experiment is shown with G data from ref. 1 in Fig. 3. It will be seen that the two sets of data agree quite well and suggest that the gravitational force law should be modified at laboratory dimensions by

$$G(R) = G_0[1 + (0.002) \ln R]$$

It should be remarked that while these results indicate an inverse square law failure at small distance, it is also clear that the experiment was carried out in the Earth's gravitational field and that we may, in fact, have a failure of the superposition principle.

I thank the many students who worked on this experiment, especially Orin Humphries, Jim Stratton, Steve Katon, Jim Daffe, Don Ogden, and Paul Horn. I also thank John Lewis for weighing the rings, Professors R. Gibbs and E. Forsman for useful conversations, and Research Corporation for support.

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- ¹ Long, D. R., *Phys. Rev.*, **D**, *9*, 850 (1974).
- ² Long, D. R., *Phys. Rev.*, **D**, *10*, 1677 (1974).
- ³ Boys, C. V., *Phil. Trans. R. Soc.*, **A1**, (1895).
- ⁴ Braun, K., *S. J. Deukschr. Akad. Wiss. Wien. Mathnaturw. Cl.* **64**, 187 (1896).
- ⁵ Rose, R. D., Parker, H. M., Lowry, R. A., Kuhlthau, A. R., and Beams, J. W., *Phys. Rev. Lett.*, **23**, 655 (1969).
- ⁶ Heyl, P., *J. Res. natn. Bur. Stand.*, **5**, 1234 (1930).
- ⁷ Heyl, P., and Chrzanowski, P., *J. Res. natn. Bur. Stand.*, **29**, 1 (1942).
- ⁸ Poynting, J. M., *Phil. Trans. R. Soc.*, **A182**, 565 (1891).
- ⁹ Richarz, F., and Krigar-Menzel, O., *Nature*, **55**, 296 (1898).

Organic complexes of iron and aluminium in natural waters

IN natural waters throughout the world¹, iron concentrations are commonly several orders of magnitude greater than the equilibrium solubility of iron hydroxide². Highly coloured waters (which have relatively high concentrations of organic matter) commonly have even greater iron concentrations. Two of the chemical species postulated to account for this phenomenon are: (1) fine colloidal particles of iron hydroxide (possibly associated with colloidal organic matter) and (2) dissolved complexes of iron with naturally occurring organic substances. While the existence of colloidal iron hydroxide in natural waters is widely accepted³, there is little conclusive evidence for or against the existence of dissolved organic complexes of iron. We wish to present evidence which suggests that dissolved organic matter forms complexes not only with iron but also with aluminium, with competition between Fe and Al for available complexing sites determining the relative abundances of the two metals in natural waters.

Lamar⁴ has shown that substantial amounts of iron and organic matter remain in solution after filtration through 0.01- μ m (100- $\text{\AA}$) membrane filters, indicating the possible presence of dissolved organic complexes of iron. Since particle diameters $< 0.01 \mu\text{m}$ have, however, been reported for colloidal iron hydroxide⁵, the results of filtration studies have thus far been inconclusive.

The frequently cited correlation between iron and organic matter concentrations⁴ suggests another possible means of distinguishing between dissolved and colloidal iron. A direct

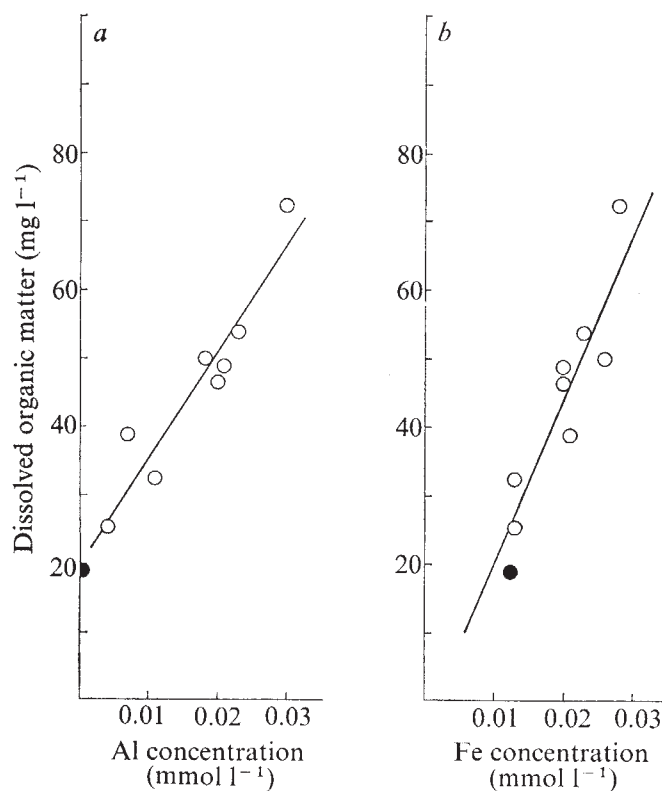


Fig. 1 Correlation between DOM (mg l^{-1}) and the concentrations of Al (a) and Fe (b) (mmol l^{-1}) for the Satilla River system ($\circ$) and for the world average river ($\bullet$). The slope for Al is $1,547 \pm 222$ (mg mmol^{-1}), intercept 20.1, and correlation coefficient 0.943. For Fe, these parameters are $2,378 \pm 472$, -2.7 , and 0.899.

correlation between iron and organic matter concentrations is more likely to result from the formation of dissolved organic complexes than from the random adsorption of colloidal iron hydroxide on the surface of colloidal organic particles. Consequently, since only a moderate correlation between iron and organic matter concentrations is usually observed in natural waters, it has generally been concluded that iron is present mainly in colloidal form. In fact, when we reported that a moderately good correlation exists between dissolved organic matter and the concentrations of iron and aluminium in the Satilla River system in the south-eastern USA, we attributed the observed scatter in the data to a lack of discrimination between dissolved and colloidal species⁸.

There is, however, another reasonable explanation for the lack of a good correlation between iron and organic matter concentrations. Since it is unlikely that the organic matter in natural waters selectively complexes iron, a substantial fraction of the available complexing sites could be used to complex other metals. Natural abundances of the various metals and stability constants of the respective metal-organic matter complexes would determine the final distribution of complexed metals. For example, because of natural variations in the local relative abundances of Al and Fe in the Satilla River system, only moderate correlations are observed between dissolved organic matter (DOM) and the individual metal concentrations (Fig. 1). An excellent correlation between DOM and the sum of Al and Fe concentrations is, however, observed (Fig. 2), indicating that the total solubility of the two metals is determined by the complexing capacity of the DOM. For comparative purposes, data for the average concentrations of DOM (19.2 mg l^{-1})⁷, Al ($0.0004 \text{ mmol l}^{-1}$)⁸, and Fe ($0.0120 \text{ mmol l}^{-1}$)¹ in the rivers of the world are also plotted in Figs 1 and 2.

Beck *et al.*⁹ have demonstrated that the bulk of dissolved organic matter in the Satilla River system closely resembles fulvic acid, an important fraction of soil organic matter which

forms stable complexes with many metal ions, including Al and Fe. In low pH, low ionic strength waters such as the Satilla River, the stability constant for the Fe-fulvic acid complex is approximately two orders of magnitude larger than the stability constant for the Al-fulvic acid complex⁹. For Al to compete successfully with Fe for available complexing sites, Al would have to be much more abundant than Fe. In the Satilla River, analyses of exchangeable cations in the $< 2 \mu\text{m}$ fraction of suspended clay minerals have shown that Al is the major metal cation and that the average Al/Fe molar ratio is > 270 (ref. 10). Thus, it is reasonable to assume that Al is present in sufficient excess to compete successfully with Fe for available complexing sites in the fulvic acid.

To calculate the molar DOM/(Al + Fe) ratio, it is necessary to estimate the molecular weight of the DOM. In a recent study, a number-average molecular weight (M_n) of 1,269 was determined by vapour pressure osmometry for DOM which was isolated from the Satilla River¹¹. This value is comparable with the M_n of 945 which has been reported for soil fulvic acids⁹. Assuming that the M_n of DOM in the present study falls

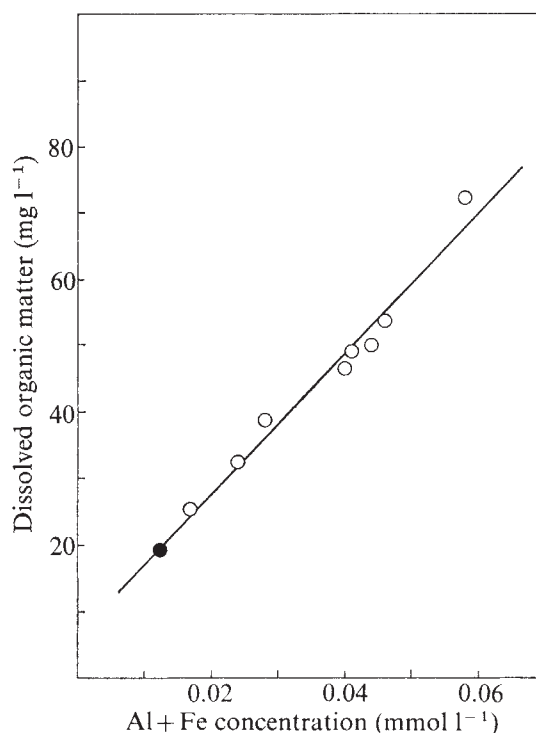


Fig. 2 Correlation between DOM (mg l^{-1}) and the sum of the concentrations of Al and Fe (mmol l^{-1}) for the Satilla River system ($\circ$) and for the world average river ($\bullet$). The slope is $1,058 \pm 78$ (mg mmol^{-1}), intercept 6.6, and correlation coefficient 0.984.

somewhere between the experimentally determined values of 945 and 1,269, the calculated molar DOM/(Al + Fe) ratio would be between 0.8 and 1.1. Thus, metal-organic complexes of $\sim 1:1$ molar ratio such as those reportedly formed in metal-fulvic acid solutions⁹ are also formed in the Satilla River.

In Fig. 2, the calculated least squares line does not pass through the origin, possibly indicating that a small fraction of the available complexing sites are occupied by metals other than Al and Fe. Based on stability constants of metal-fulvic acid complexes⁹, Ca is the only major cation which might be complexed to a significant extent. Although the Ca/(Al + Fe) ratio is $\sim 1:1$ in the Satilla River⁶, a value of 30:1 is observed in the world average river¹. Thus, in the world average river, a substantial fraction of the complexing sites may be occupied by Ca, leaving much of the total Al and Fe present as colloidal

hydroxides. Thus, it is probably fortuitous that the ratios of DOM to Al, Fe and (Al + Fe) in the world average river are the same as those found in the Satilla River (see Figs 1 and 2). Only when actual data are available for other river systems will it be possible to determine whether the metal-organic interactions observed in the Satilla River are generally important in the transport of iron and aluminium in natural waters.

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- ¹ Livingstone, D. A., *U.S. geol. Surv. Prof. Pap.* 440-G (1963).
- ² Stumm, W., and Morgan, J. J., *Aquatic Chemistry*, 238-299 (Wiley, New York, 1970).
- ³ Hem, J. D., and Cropper, W. H., *U.S. geol. Surv. Water Supply Pap.* 1459-A (1959).
- ⁴ Lamar, W. L., *U.S. geol. Surv. Prof. Pap.* 600-D, D24-D29 (1968).
- ⁵ Lengweiler, H., Buser, W., and Feitknecht, W., *Helv. chim. Acta*, **44**, 805-811 (1961).
- ⁶ Beck, K. C., Reuter, J. H., and Perdue, E. M., *Geochim. cosmochim. Acta*, **38**, 341-364 (1974).
- ⁷ Garrels, R. M., and Mackenzie, F. T., *Evolution of Sedimentary Rocks*, **101** (Norton, New York, 1971).
- ⁸ Sackett, W. M., and Arrhenius, G. O. S., in *Preprints Int. Oceanogr. Congr.* (edit. by Sears, M.), 824-825 (American Association for the Advancement of Science, Washington, 1959).
- ⁹ Schnitzer, M., and Hansen, E. H., *Soil Sci.*, **109**, 333-340 (1970).
- ¹⁰ Beck, K. C., *Completion Report OWRP Project B-033-GA* (Environmental Resources Center, Georgia Institute of Technology, 1972).
- ¹¹ Martin, S. J., and Reuter, J. H., *Abstracts with Programs*, **5** (7), 727 (Geological Society of America, Boulder, Colorado, 1973).

Shortening of growing season in the US corn belt

I ATTEMPT here to relate the gradual and oscillatory temperature drop in the Northern Hemisphere to a shortening of the length of the growing season in the US corn belt.

The Northern Hemisphere has cooled down during the past 30 yr (refs 1-3); Kukla and Kukla² have shown that the increase in the mean annual area of snow and ice over the Northern Hemisphere during the winter of 1971-72 was equivalent to one-sixth of the change necessary to bring back the full-ice

conditions of the most recent ice age. Wahl and Bryson³ reached a similar conclusion, and stated that in the past 30 yr the sea-surface temperatures measured at nine ship stations in the North Atlantic have decreased by an amount equivalent to one-sixth of the drop to minimum ice age temperatures of 17,000 yr ago. Similarly, the average length of the growing season at six climatological stations with the longest meteorological records in the US corn belt (see Fig. 1) have shortened by 14% (27 d) within the past 30 yr. Detailed study of corn belt data, however, indicates highly oscillatory year-to-year variations over the past 75-100 yr, indicating the need for caution in predicting trends.

The six climatological stations covered in Fig. 1 are located to the south and south-west of the Great Lakes. The prevailing winds are generally south-westerly during the growing season and there is great geographical variability of the observed parameters within the area. The microclimatology of some stations is affected by riverine or urban and industrial influences. For that reason I have not attempted to establish a representative average change in the growing season in the corn belt.

The growing season at Toledo (Fig. 1a) decreased by as much as 43 d during the last 30 yr, the largest decrease of the six stations. On the other hand, no change, or a slight increase, was observed at Peoria (Fig. 1d), a site affected by both riverine and urban and industrial influences. Except for Peoria, the decrease in the length of the growing season at the remaining five sites varies between 22 and 39 d (Fig. 1).

The growing season is defined as the number of days between the last killing frost in the spring and the first in the autumn. It is important to realise, however, that though the length of the growing season is a significant factor, other climatic variables also impose significant limits on corn production. Thompson has stated⁴ that the unusually high temperatures from 1930 to 1940, especially during the months of July and August, resulted in below average crop yields compared with those expected during normal weather. After 1940, although the length of the growing season in the corn belt was generally decreasing, both temperature and moisture were ideally distributed, and record corn yields were established. As the average growing season in the corn belt is now 165 d little further decrease can be tolerated as the minimum requirement for hybrid corn is 100-130 d. A more important factor is the ever present, large, year-to-year, oscillatory character of the growing season: for example, the abnormally early killing frosts in Iowa in 1875 (August 22), in 1950 (August 20), and in 1974 (September 5).

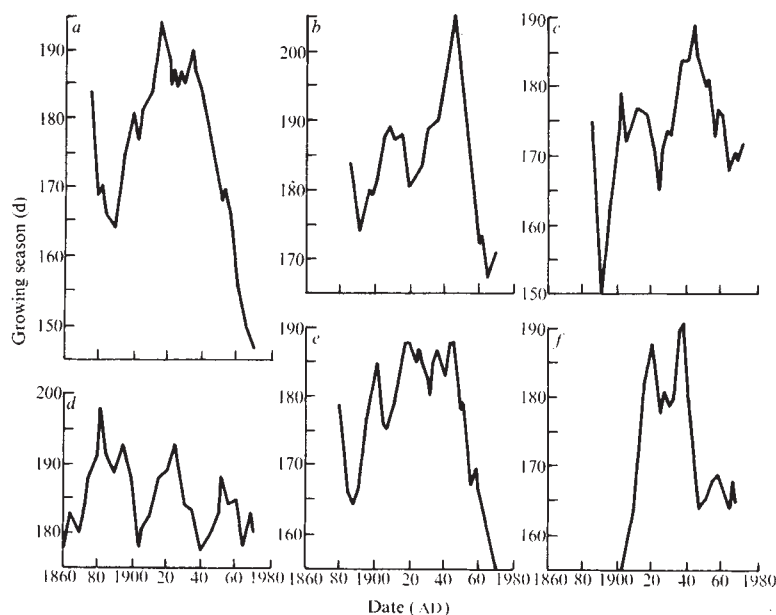


Fig. 1 11-yr running averages of the variations in the length of the growing season for six stations in the US corn belt: a, Toledo; b, Columbus; c, Des Moines; d, Peoria; e, Dubuque; f, Fort Wayne.

The variations in the length of the growing season seem to serve as a useful climatic index, directly related to the agricultural production areas and sensitively linked to the variations of the seasonal patterns.

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¹ Mitchel, J. M., Jr., *Ann. N.Y. Acad. Sci.*, **95** (1), 235–250 (1961).

² Kukla, G. J., and Kukla, H. J., *Science*, **183**, 709–714 (1974).

³ Wahl, E. W., and Bryon, R. A., *Nature*, **254**, 45–46 (1975).

⁴ Thompson, L. M., *Agron. J.*, **61**, 453–456 (1969).

Entropy of vitreous ice

THE excess thermodynamic properties of vitreous solids over those of crystalline forms include contributions from two sources: first, the configurational changes that occur in the vitreous state¹; and, second, lattice vibrations that are of a relatively low frequency and are thought to be relatively more anharmonic. We consider here the excess and the residual entropy of vitreous ice and discuss each in terms of the molecular processes and the structure.

There are at least two allotropic forms of vitreous ice², obtained by depositing vapours on a substrate maintained at 10 K in one case, and at 77 K in the other. The former has a density nearly 20% higher than the latter, and its oxygen pair correlation function obtained from X-ray diffraction shows an additional peak at 0.33 nm, corresponding to about 1.4 next near-neighbours². It is also noteworthy that very thin deposits of amorphous films of ice on cold (< 173 K) thimbles of hygrometers³—a common and unwanted occurrence in meteorological experiments—may be yet another allotropic form. Only vitreous ice, obtained by depositing vapours on a substrate held at 77 K, with a 'glass transition temperature', T_g , of 135 K, is considered here.

The entropy, S , of vitreous, cubic and hexagonal ice is plotted against temperature in Fig. 1. The values were obtained from the known heat capacity of vitreous and cubic ice⁴ at temperatures above 21 K, and from the calculated heat capacity below 21 K, assuming a constant Debye temperature. The entropy of hexagonal ice was obtained from the known heat capacity at temperatures above 2 K (refs 5–7). The excess entropy, S_{exc} , which is the difference between the measured values ($S_{vitreous} - S_{crystal}$), is also plotted against temperature in Fig. 1.

At high temperatures the vibrational heat capacity of solids approaches the classical limit, k , per degree of freedom. If the lattice vibrations were solely responsible for the difference between the entropies of the amorphous and crystalline solid, a plot of S_{exc} against temperature would resemble the Debye heat capacity curve; that is, it would show a rapid rise at low temperatures followed by an approach to an asymptotic value at a characteristic temperature, θ , given by, $\theta = hv/k$, of the frequencies, ν , that differ between the two solid forms.

The S_{exc} value of vitreous ice follows the Debye behaviour up to 85 K (Fig. 1). It shows a rapid rise in the range 5–40 K, followed by a nearly constant value of $1.2 \text{ J K}^{-1} \text{ mol}^{-1}$ in the range 55–85 K. Above 85 K, S_{exc} increases progressively with temperature and reaches a value of $2.1 \text{ J K}^{-1} \text{ mol}^{-1}$ at 135 K ($= T_g$), at which the spontaneous crystallisation to cubic ice occurs. The temperature dependence of S_{exc} in Fig. 1 is remarkably similar to that observed for vapour-deposited solid methanol, for selenium and other glasses⁸ and for the disorder frozen-in state of crystals⁹.

The initial increase in S_{exc} of $1.2 \text{ J K}^{-1} \text{ mol}^{-1}$ is partly attributable to the difference in the Debye frequencies and partly to the difference in the degree of anharmonicity of lattice

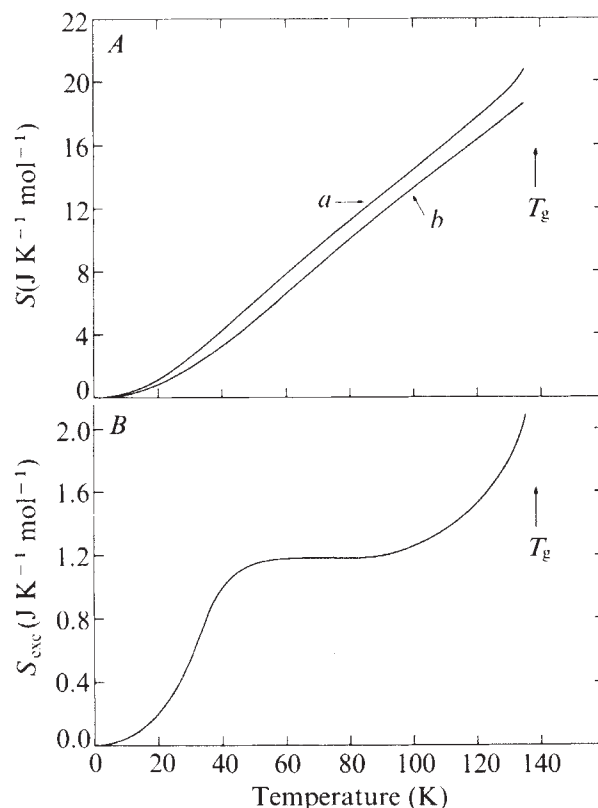


Fig. 1 A, Calorimetric entropy of vitreous (a), and cubic and hexagonal (b) ice plotted against temperature in the top half. Within the experimental error of 1%, the entropy of cubic and hexagonal ice are the same below 135 K. B, Excess entropy of vitreous ice over crystalline ice ($S_{vitreous} - S_{crystal}$) plotted against temperature. T_g , The 'glass transition temperature' at which spontaneous crystallisation to cubic ice also occurs.

vibrations. It is considerably smaller than the initial increase found for other amorphous solids^{8,9} but seems reasonable in view of the fact that vitreous and hexagonal ice have nearly the same molar volume² and the same number of near neighbours at a distance of 0.276 nm.

The 75% increase in S_{exc} between 85 K and 135 K is no doubt attributable to the presence of configurational degrees of freedom, and indicates the onset of small-scale molecular rearrangements in vitreous ice. These may occur in some local regions surrounded by a rigid amorphous matrix, as in many molecular glasses¹, and would involve either or both of the rotational and translational motions of water molecules. These molecular motions would show up in a dielectric study as either a secondary relaxation¹ or a high value of the dielectric loss.

The residual entropy, S_{res} , of vitreous ice must, of course, have contributions from two types of disorder of water molecules; one, the positional and the other, the orientational. The contribution from the positional disorder can be readily inferred from a comparison with an analogous tetrahedrally-bonded structure. Since the continuous random network structure of vitreous ice¹⁰ is similar to that of vitreous silica¹¹, the maximum value of this contribution is $5.8 \text{ J K}^{-1} \text{ mol}^{-1}$, a value calculated for the latter from topological considerations¹². The maximum contribution to S_{res} from orientational disorder is $3.4 \text{ J K}^{-1} \text{ mol}^{-1}$ ($= R \ln(3/2)$), as in hexagonal and cubic ice, but a realistic value would be less, for, in the vitreous state, the six orientations of a given water molecule would not be equally probable and consequently, each of the $(3/2)^N$ configurations would not have the same energy. The maximum theoretical value of S_{res} , anticipated for a fully H-bonded, fully (orientationally) disordered, vitreous ice is, therefore, $9.2 \text{ J K}^{-1} \text{ mol}^{-1}$.

The calorimetric value of S_{res} of vitreous ice cannot be obtained from the Third Law of Thermodynamics as the course from vitreous ice to the equilibrium liquid state at 273 K

involves two irreversible transformations: first, vitreous $\rightarrow$ cubic ice at 135 K, and, second, cubic $\rightarrow$ hexagonal ice in the range 160–210 K. Only the maximum value of calorimetric S_{res} can be obtained using the Clausius inequality principle. Thus: $dS > (dq/T)$, where dq is the heat evolved in the process at a temperature T . For the transformation, vitreous $\rightarrow$ cubic ice, $dq = -1.64 \text{ kJ mol}^{-1}$ at $T = 135 \text{ K}$ (ref. 4). Therefore, dS , which is the minimum entropy loss at the transformation, is $12.1 \text{ J K}^{-1} \text{ mol}^{-1}$. The S_{res} can be written as

$$(S + S_{res})_{\text{vitreous}} - (S + S_{res})_{\text{cubic}} < 12.1 \text{ J K}^{-1} \text{ mol}^{-1}$$

At 135 K, $S_{\text{vitreous}} = 20.8$, $S_{\text{cubic}} = 18.7$ and $(S_{res})_{\text{cubic}} = 3.4 \text{ J K}^{-1} \text{ mol}^{-1}$. The maximum value of the calorimetric S_{res} is, therefore, $13.4 \text{ J K}^{-1} \text{ mol}^{-1}$, which is consistent with the corresponding theoretical value of $9.2 \text{ J K}^{-1} \text{ mol}^{-1}$.

The agreement between the maximum value of the theoretical and the calorimetric S_{res} is significant, although it does not indicate whether an increase in S_{res} , such as would occur if the vitreous ice were not completely H-bonded, is partly compensated for by a decrease in S_{res} attributable to the lack of complete orientational disorder. In the absence of the heat capacity data of liquid water between 135 and 230 K, this analysis seems to be the simplest and perhaps most appropriate.

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- 1 Johari, G. P., *J. chem. Phys.*, **58**, 1766–1770 (1973).
- 2 Wenzel, J., Linderström-Lang, C. U., and Rice, S. A., *Science*, **187**, 428–430 (1975).
- 3 Dobson, G. M. B., Brewer, A. W., and Cwilong, B. M., *Proc. R. Soc.*, **A185**, 144–175 (1946).
- 4 Sugasaki, M., Suga, H., and Seki, M., *Bull. chem. Soc. Japan*, **41**, 2591–2599 (1968).
- 5 Giauque, W. F., and Stout, J. W., *J. Am. chem. Soc.*, **58**, 1144–1150 (1936).
- 6 Flubacher, P., Leadbetter, A. J., and Morrison, J. A., *J. chem. Phys.*, **33**, 1751–1755 (1960).
- 7 Haida, O., Matsuo, T., Suga, H., and Seki, S., *J. chem. Thermodyn.*, **6**, 815–825 (1974).
- 8 Goldstein, M., *Ann. N.Y. Acad. Sci.* (in the press).
- 9 Johari, G. P., *Ibid* (in the press).
- 10 Alben, R., and Boutron, P., *Science*, **187**, 430–432 (1975).
- 11 Zachariasen, W. H., *J. Am. chem. Soc.*, **54**, 3841–3851 (1932).
- 12 Bell, R. J., and Dean, P., *Phys. Chem. Glasses*, **9**, 125–127 (1968).

Restricted pollen flow of two woodland herbs determined by neutron-activation analysis

THE movement of pollen is a critical event in the population genetics of wind-pollinated plants. Neighbourhood size and potential for genetic differentiation, for example, are significantly influenced by pollen flight distances^{1,2}. The evolution of phenological and morphological mechanisms that enhance wind pollination is well studied^{3,4}, but the actual distance of pollen flight in natural populations is less clearly documented. Pollen movements have been estimated by using genetic markers in field plots⁵, trapping pollen with artificial barriers⁶, and catching radioisotope- and chemically-marked pollen of conifers^{7–9}. I report the successful measurement of wind-dispersed pollen in natural populations of woodland herbs, using neutron-activation analysis of surface-treated pollen. Preliminary results suggest highly restricted pollen movement in these herbs.

Carex plantaginea Lam. and *Carex platyphylla* Carey (Cyperaceae) are common sedges in New York forests. These monoecious plants flower in early May. The florets are highly modified for wind pollination, although the different culm (stem) morphologies of the two species suggest

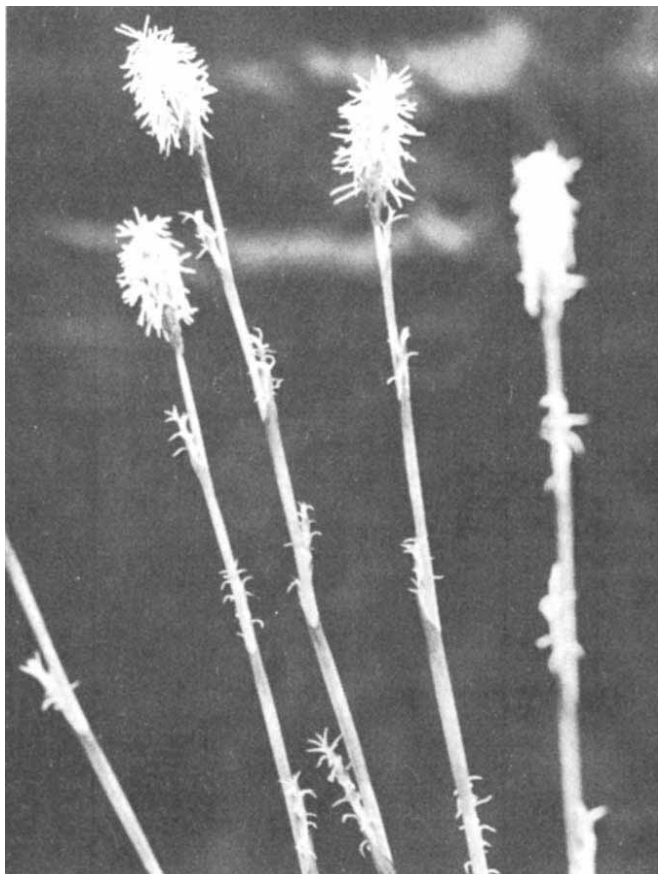


Fig. 1 Tops of fertile culms of *Carex plantaginea* during anthesis. Lateral pistillate spikelets have exerted stigmas a few days before and during opening of anthers from the terminal staminate spikelet. *Carex platyphylla* has a similar inflorescence, but is shorter ($\sim 12 \text{ cm}$ as against 20 cm for *C. plantaginea*), with lateral spikelets more clustered towards the apex, and erect foliose bracts subtending the lateral spikelets. ($\times 0.39$).

that pollen flow might be relatively lower in *C. platyphylla*. Fertile culms elongate well before most other vernal herbs appear; thus, there are few herbaceous barriers to pollen flight.

Pollen movements within a population of each species were estimated by marking pollen of selected plants with a normally absent element, samarium. After pollen dispersal, traces of samarium were searched for on stigmas of plants throughout an area uninterrupted by trees. A section of a local population of each sedge species was selected for study. These had similar densities, ~ 15 plants per m^2 . Before exertion of anthers, $15 \mu\text{l}$ (*C. platyphylla*) or $25 \mu\text{l}$ (*C. plantaginea*) of a samarium solution were applied with syringes to staminate spikelets of several central culms in each cluster. A 0.1 M solution of samarium oxide of natural isotopic abundance was prepared in hot dilute HNO_3 (ref. 10). This was buffered with 0.1 M EDTA, then the pH was raised to 5.7 with 1 M NaOH. For use on these species, a 0.15% solution of Tween 20 (polyoxyethylene [20] sorbitan monooleate) was added as a wetting agent.

The day after anthesis, pistillate spikelets from both inoculated plants and others in the cluster were collected into washed polyethylene vials. The wind speed was normal, $< 10 \text{ km h}^{-1}$, on days of pollen flight. Spikelets from three culms at each sample point were put in each vial, and a control series of vials was filled with *C. plantaginea* spikelets before any samarium was introduced into the population. Neutron-activation analysis of samples followed, to determine the amount and presence of samarium-labelled pollen at the various distances from the treated plants. For this analysis, the samples were bombarded with neutrons, causing unstable isotopes of samarium to be formed. The

major activity comes from 47-h ^{153}Sm which β decays to ^{153}Eu , which in turn emits γ rays. The dominant emissions, 70 keV and 104 keV γ rays¹¹, were counted on a NaI scintillation detector. Data are summarised in Table 1.

These data record a rapid decrease of activity within short distances from samarium-treated spikelets. Ten readings from pistillate spikelets from the samarium-treated culms of *C. platyphylla* show great activity. Within 0.15 m from the inoculated culms, there is a greater than 30-fold decrease of activity. By 0.5 m from the treated spikelets, activity at some locations was halved again, and many locations recorded no activity above control levels. Beyond 1.0 m, only four readings showed activity above the control. No bias in directionality of pollen flow was seen in these data. (No samples from the central, inoculated *C. plantaginea* culms were available, as these particular culms failed to develop mature pistillate florets, a common occurrence in population of this species¹³.)

The generally lower dispersal range of *C. platyphylla* pollen supports the hypothesis that the culm morphology of this species lowers pollen flow. This may be attributed in part to the lower height of the staminate spikelets on these culms. Dispersal distance varies inversely with height of pollen source¹⁴. Also, the subtending foliose bracts beneath *C. platyphylla* pistillate spikelets act as mechanical barriers to pollen.

Additional studies of pollen movement in these species might include flow rate throughout clusters of similar density, but containing tree trunks. Such clusters would be expected to have lower dispersal levels, as tree stands decrease pollen movement^{15,16}. Also, information on congruence of movements of treated and untreated pollen, flow during high wind speeds, and correlation between pollen movement and actual gene flow are needed. Even if infrequent, long distance pollen flow could be important to genetic processes.

The wind dispersal pattern in this study is consistent with the leptokurtic pattern seen in studies using other methods. Similarly, highly restricted pollen flow is reported from many insect-pollinated populations³. The neutron-

activation analysis results support evidence that herb populations may be quite isolated genetically from adjacent populations¹⁷, and that local population differentiation over the range of a species may be frequently achieved¹⁸⁻²¹. The low pollen dispersal pattern of these *Carex* species superficially adapted for 'wide' pollen flow, highlights the need for care in postulating large neighbourhood size in their populations.

The use of neutron-activation techniques for studies on genetic structure and processes in populations is recommended for its relative ease and accuracy^{22,23}. This technique is also applicable to other ecological studies by field biologists, such as recording visitation patterns of nocturnal pollinators, feeding behaviour of nectar feeders, or occurrence of food exchanges between members of animal colonies.

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- ¹ Wright, S., *Ann. Eugen.*, **15**, 323-354 (1951).
- ² Levin, D. A., and Kerster, H. W., *Evol. Biol.*, **7**, 139-220 (1974).
- ³ Whitehead, D. R., *Evolution*, **23**, 28-35 (1969).
- ⁴ Proctor, M., and Yeo, P., *The Pollination of Flowers*, (Collins, London, 1973).
- ⁵ Griffiths, D. J., *J. agric. Sci., Camb.*, **40**, 19-38 (1950).
- ⁶ Raynor, G. S., Ogden, E. C., and Hayes, J. V., *J. appl. Meteorol.*, **9**, 885-895 (1970).
- ⁷ Colwell, R. N., *Am. J. Bot.*, **38**, 511-523 (1951).
- ⁸ Fendrik, I., and Glubrecht, H., in *Nuclear Activation Techniques in the Life Sciences*, 325-331 (International Atomic Energy Agency, Vienna, 1967).
- ⁹ McElwee, R. L., thesis, North Carolina State Univ. (1970).
- ¹⁰ Gaudreau, M. M., and Hardin, J. W., *Brittonia*, **26**, 316-320 (1974).
- ¹¹ Lederer, C. M., Hollander, J. M., and Perlman, I., *Table of Isotopes*, 6th ed. (Wiley, New York, 1967).
- ¹² Heath, R. L., *Scintillation Spectrometry* (US Atomic Energy Commission, 1964).
- ¹³ Handel, S. N., thesis, Cornell Univ. (1974).
- ¹⁴ Sutton, O. G., *Q. Jl R. met. Soc.*, **73**, 426-436 (1947).
- ¹⁵ Dengler, A., *Z. Forstgenet. Forstpf.-Zücht.*, **4**, 107-110 (1955).
- ¹⁶ Raynor, G. S., *Nucl. Sci. Abstr.*, **22**, 4100 (1968).
- ¹⁷ Ehrlich, P. R., and Raven, P. H., *Science*, **165**, 1228-1232 (1969).
- ¹⁸ Aston, J. L., and Bradshaw, A. D., *Heredity*, **21**, 649-664 (1966).
- ¹⁹ McNeilly, T., *Heredity*, **23**, 99-108 (1968).
- ²⁰ Snaydon, R. W., *Evolution*, **24**, 257-269 (1970).
- ²¹ Schaal, B. A., *Am. Nat.*, **109**, 511-528 (1975).
- ²² Bowen, H. J. M., in *Nuclear Activation Techniques in the Life Sciences*, 287-297 (International Atomic Energy Agency, Vienna, 1967).
- ²³ Richardson, R. H., Wallace, R. J., Gage, S. J., Bouchey, G. D., and Dennell, M., *Univ. Tex. Publs Stud. Genet.*, **5**, 171-186 (1969).

Inhibition of float formation in water hyacinth by gibberellic acid

THE water hyacinth (*Eichhornia crassipes*) is one of the most harmful weeds of tropical and subtropical waterways and lakes. Its reproductive potential is enormous and in favourable conditions it forms dense mats which may cover the surface of a body of water. Such prolific development can obstruct the drainage of farmlands, block hydroelectric installations in dams, hinder navigation and affect fisheries (reviewed in ref. 1). The leaves of free-floating water hyacinths are characterised by a bulbous swelling in a section of the petiole which provides buoyancy. These floats, however, do not develop when the plants are rooted in the soil of muddy shores. In those circumstances the petiole narrows gradually from its base to the leaf blade. We have found that the formation of floats can be inhibited by extremely low concentrations of gibberellic acid (GA_3) in the water. As a consequence of treatment the plants become unstable and partially sink below the surface of the water.

Water hyacinth plants were cultured *in vitro* in polyethylene trays (45 × 30 × 8 cm) on 1/5 strength Long Ashton medium composed of the following (mg per l of nutrient solution):

KNO_3 , 81; $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 189; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 74;
 $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 0.35; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.35; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.05;

Table 1 Relative amount of samarium-treated pollen at distances from treated staminate spikelets

Distance (m)	<i>C. platyphylla</i>		<i>C. plantaginea</i>		Activity of B (Mean s.d.)	
	A*	B†	A	B		
0.00	10	10			1,498	504
0.15	4	4	2	2	44	20
0.20	12	0	6	2	56	16
0.30			2	2	27	4
0.40	8	0				
0.50	8	0	6	2	22	1
0.60	4	1			36	
0.70	2	0				
0.75			2	0		
0.90	2	0	2	0		
1.00			6	3	32	11
1.10	2	0				
1.25			2	0		
1.35	2	0				
1.50			2	0		
2.50			2	0		
3.00			2	0		
3.20			4	2	19	5
3.75			2	2	23	7
5.00			4	0		

*A, Total number of peaks counted.

†B, Number of peaks with activity > 1 s.d. above mean of the control series.

Samples were irradiated at the Cornell TRIGA MARK II reactor for 1 h with a neutron flux in the range of 7×10^{11} neutrons cm^{-2} s^{-1} . After 18-h decay, γ -spectrum analysis was done with a 3" × 3" NaI (TI) well-counter (Baird-Atomic Model 810C) and a 256-channel pulse-height analyser (Technical Measurement Corp. Model CN-110). Scintillations were counted for 120-600 s. Counts were calculated from 40 channel widths, relativised for count time, and corrected for decay and background radiation¹².

$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.06; H_3BO_3 , 0.6; NaCl , 1.2;
 $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 0.02; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 5.6; EDTA, 5.8

adjusted to pH 7 with 1 N NaOH. The plants were exposed to continuous illumination ($12,000 \text{ erg cm}^{-2} \text{ s}^{-1}$) from white fluorescent tubes at $27 \pm 2^\circ \text{C}$. Experiments were conducted in a greenhouse during the summer in reservoirs ($100 \times 100 \times 60 \text{ cm}$) containing 20 cm of pot soil and filled up with tap water. The circumference of the floats and the length of the petioles were measured with a sliding gauge with Vernier scale (accuracy 0.1 mm). All experiments lasted 4 weeks. When the plants were cultured *in vitro* typical float leaves were formed (Fig. 1a). Increasing concentrations of GA_3 , however, decreased the diameter of floats of newly formed leaves. As little as 0.03 p.p.m. of GA_3 (0.03 mg l^{-1}) in the nutrient medium suppressed the formation of float leaves (Fig. 1b). Even with 0.01 p.p.m. of GA_3 the floats were clearly smaller than those of controls. In Fig. 2 the quotient of petiole length and greatest circumference (l/c) is presented at different concentrations of GA_3 . These values decrease markedly at concentrations of 0–0.03 p.p.m. At the highest concentration tested, 1.0 p.p.m., the value was very high because of the considerable increase in the length of the petiole. GA_3 also markedly inhibited vegetative multiplication of the plants and induced profuse flowering. 6-Benzyladenine (BA) counteracted the effect of GA_3 . In the presence of BA the floats became slightly thicker than in the controls and plants with elongated leaves which had been cultivated on GA_3 -containing medium reproduced floats quicker in the presence than in the absence of BA.

2-Chloroethyltrimethylammonium chloride (CCC), indol-3-yl-butyric acid (IBA), abscisic acid and the ethylene-releasing

Fig. 1 a, Water hyacinths after cultivation on 1/5 strength Long Ashton medium. Note the typical float leaves. b, As (a) but supplemented with 0.03 p.p.m. GA_3 . Note the absence of floats in the newly formed leaves.

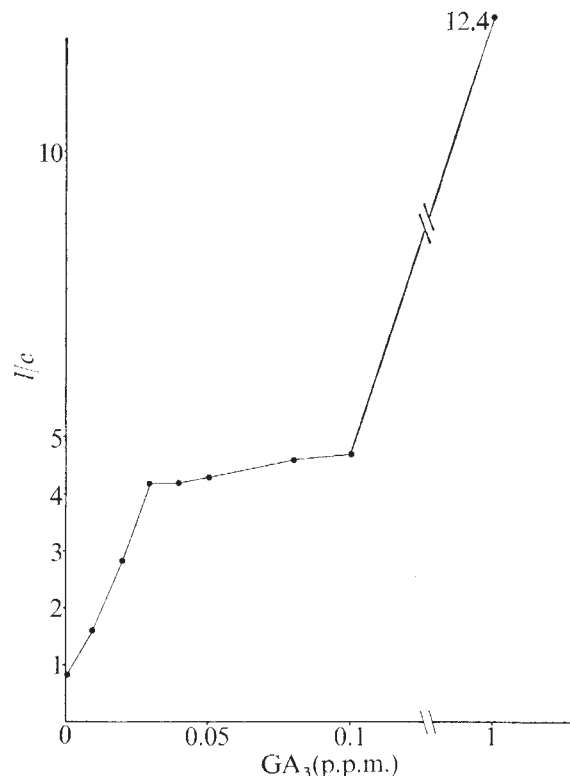


Fig. 2 Effect of various concentrations of GA_3 on float formation in water hyacinth, expressed as the quotient of length (l) and circumference at the greatest width (c) of the petiole.

compound ethrel had no effect on float formation. Plants grown in the greenhouse in the presence of 0.03 p.p.m. of GA_3 produced only elongated leaves. These plants produced just a few daughter plants and eventually became submerged. The control reservoirs, on the other hand, were completely overgrown within 4 weeks. Preliminary spray tests in the greenhouse have shown promising results in the inhibition of float formation by GA_3 .

In the wild, water hyacinths do not produce float leaves in crowded conditions or when rooted in the mud². It has also been reported that a relatively high temperature and low light intensity (in the shade) promote elongation of the petiole³. Our results suggest that the external factors which influence float formation in water hyacinth act through endogenous gibberellins and perhaps cytokinins. The effective concentration of GA_3 (0.03 p.p.m.) was extremely low and as this hormone markedly inhibits vegetative growth it could perhaps be used to control the growth of water hyacinths in certain areas. It might be combined with biological means of control, such as *Neochetina eichhorniae* (water hyacinth beetle) or *Orthogalumna terebrantis* (water hyacinth mite). The very low concentrations of GA_3 that were effective would be economically feasible, and as GA_3 is a natural component of plants it should not endanger the environment.

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¹ Pieterse, A. H., *Trop. Abstr.*, 29, 77–92 (1974).

² Penfound, W. T., and Earle, T. T., *Ecol. Monog.*, 18, 447–472 (1948).

³ Boresch, K., *Flora, Jena*, 104, 296–308 (1912).

Morphogenetic mutants detected in mitotic recombination clones

GENETIC analysis has been used successfully for identifying the elements responsible for biological processes^{1,2}, and understanding the logic of their interactions. This approach has, however, been little used in the study of morphogenesis in multicellular organisms, chiefly because of a dearth of viable mutants and the difficulties of interpreting the altered primary gene function from the mutant phenotype. The existence of pleiotropy in particular makes this analysis difficult^{3,4}. An alternative approach would be to study the effect of mutations on individual cell behaviour⁵. This approach depends on the well founded assumption that normal morphogenesis results from the ordered integration of discrete cell autonomous functions. This can be done in *Drosophila* in genetic mosaics, where the heterozygous, phenotypically wild-type individual is a carrier of clones of homozygous mutant cells. This approach has the advantage that morphogenetic mutants, even if they are lethal to the individual, can be detected, studied and interpreted directly at the cellular level.

Previous analysis in *Drosophila* has shown that about 90% of loci can mutate to a lethal condition^{6,7}. Some 90% of these lethals are, however, viable in clones of epidermal cells and therefore amenable to clonal analysis⁸. Once the normal clonal parameters of a given developmental system are known⁹⁻¹¹, it is possible to detect mutants altering them. Here we describe a method of directly detecting and characterising morphogenetic mutants in clones produced by mitotic recombination. A similar approach has been used to detect cuticular differentiation mutants¹².

Morphogenetic mutants, induced in a chromosome arm carrying cell marker mutants, can be detected after mitotic recombination as morphological abnormalities associated with the cell marker phenotype. If the mitotic recombination event removes at the same time the *Minute* (*M*) allele in heterozygous *M/M*⁺ growing cells, the resulting clones will be large¹³ enough to be seen under the dissecting microscope. This procedure enables us to uncover mutants (1) in the chromosome arm of

Fig. 1 *mwh jv* * *M(3)i*⁵⁵⁺ notum showing intercalary addition of chaetal elements.

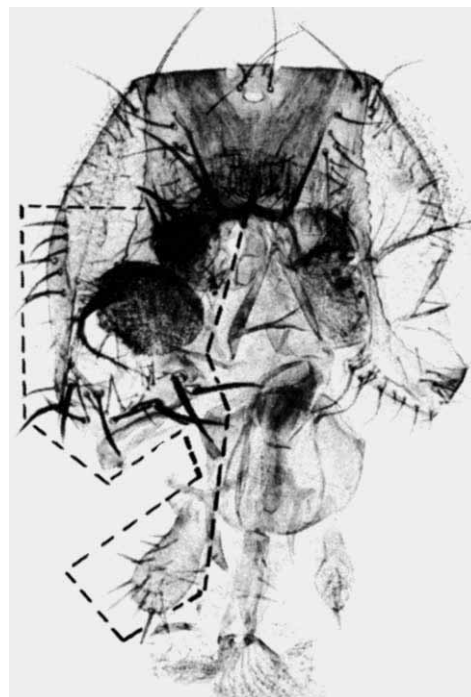


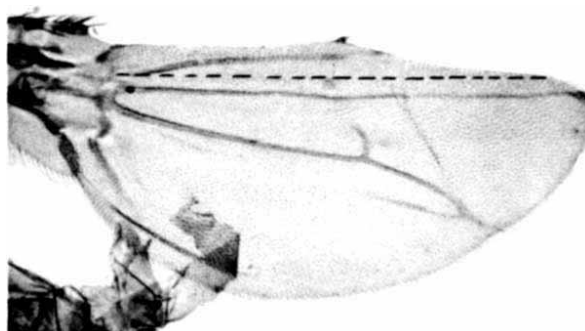
Fig. 2 *mwh jv* * *M(3)i*⁵⁵⁺ head showing cuticular elements about twice as large as wild type.

the *Minute*, (2) in heterozygous condition, (3) irrespective of whether they are lethal or present in a lethal-carrying chromosome and (4) as autonomous as well as partially nonautonomous cell mutations.

Thus, male gametes of the genotype *mwh jv*, mutations which mark the trichomes and chaetes respectively¹⁴, were treated with ethylmethane sulphonate (EMS) (0.02 M)¹⁵. The treated males were crossed to balancer-carrying females (*TM1,sbd/TM3,e*¹¹) and the *F*₁ *mwh jv**/*TM1* and *mwh jv**/*TM3* females mass-crossed to *M(3)i*⁵⁵ *sbd/TM1* or to *M(3)i*⁵⁵ *e*¹¹/*TM3* males respectively. The *F*₂ progeny was irradiated with 500-r. X rays at 48–72 h after oviposition¹⁶. The emerged *mwh jv**/*M(3)i*⁵⁵ males were then scored under the dissecting microscope for morphological abnormalities, which could be anywhere in the body cuticle. Those males showing an abnormality were then crossed to balancer-carrying females, to ensure amplification of the treated chromosome, and then mounted on a slide and checked for the existence of *mwh jv* *M*⁺ territories associated with the morphological abnormality. In positive cases the existence of a morphogenetic mutant was again verified in mitotic recombination clones in subsequent generations, mapped meiotically, separated from associated lethals by recombination, and maintained in balanced stocks for detailed study of its phenotype.

Out of 20 EMS-independent treatments, about 10,000 males of *mwh jv**/*M(3)i*⁵⁵ genotype were scored under the dissecting

Fig. 3 *mwh jv* * *M(3)i*⁵⁵⁺ wing lacking most of the anterior region. Some *mwh* cells remain.



microscope. Morphological abnormalities were detected in 95 individuals but progeny were obtained only from 68 of them. In 25 of the 68 individuals the malformation was associated with the *mwh jv* M^+ genotype, but in only 19 cases did the original malformation appear in subsequent generations. The association of the malformation with the clone of cell marker mutants indicated that it corresponds to a true mutation in the left arm of the third chromosome. Through further analysis we succeeded in assigning to each malformation a locus in that chromosome arm. All the isolated mutants have been shown to be lethal or closely linked to a lethal mutation. We tested the 19 mutants isolated for allelomorphism and found two pairs which did not complement. One of these pairs occurred in flies subjected to the same EMS treatment and can be attributed to the same mutational event and another pair corresponded to true alleles.

The different clonal phenotypes of these 18 mutants were classified as follows. A first group of five mutants lack entire regions of the adult cuticle, the surrounding territories being of more or less normal morphology (Fig. 3). They differ depending on the degree of autonomy of the lethal effect, extension of remnant *mwh jv* cells still present at the borders of the clone, and on the effect on the surrounding wild-type cells.

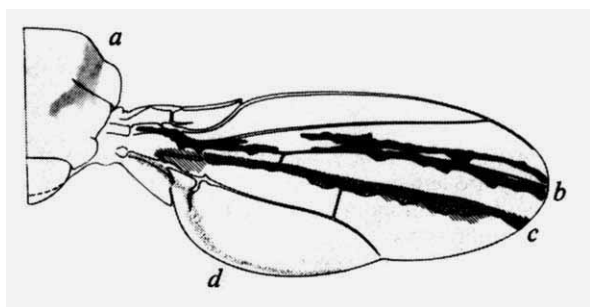


Fig. 4 *mwh* * $M(3)^{55+}$ Diagram representing four elongated and split clones; a, b and c correspond to one mutant and d to another. Clone c runs along the antero-posterior compartment border in both dorsal and ventral surfaces of the wing.

A second group of nine mutants modifies the pattern of the clone in different ways. In two different alleles of the same locus the adult cuticular elements (trichomes and chaetae) are about twice as large as for the wild type (Fig. 2). Another mutant leads to the intercalary addition (2–3 times) of chaetae in an otherwise normally patterned tissue (Fig. 1). Two mutants lead to the formation of elongated and split clones, which are smaller than control ones of the same age (Fig. 4). Two mutants lead to the formation of outgrowths in the cuticular surface, one associated with whirls in the orientation of the trichomes and chaetae, and the other with thick and dark, poorly differentiated cuticle. A possible homoeotic mutant shows a transformation of the third joint of the antenna into tissue typical of the proboscis; clones in other regions of the body are of abnormal appearance.

A third group of five mutants shows different types of abnormality in the cuticle associated with chaetae and trichome pigmentation and/or morphology. They can be considered as mutations that affect the mechanisms of cuticular formation¹².

The mutant phenotypes described are suggestive of as many cell functions as can be discriminated by mutation. They possibly result from alterations in the mechanisms of growth and response to metamorphosis, cuticular differentiation, and in the control of sequential mitotic orientations, of the size of cuticular elements or of their distribution in patterns, and so on. From analysis of these and other mutants detected in this way we hope to infer the wild-type functions relevant to the genetic control of morphogenesis. These phenotypes examined at the cellular level, will also help to suggest molecular mechanisms underlying such cellular abnormalities. The

efficiency of the reported method of detection of morphogenetic mutants is very high: about 1 such mutation for 500 flies scored. Using cell marker mutants¹² and *Minutes*¹⁶ in other chromosome arms it will soon be possible to extend these studies to mutants over the entire *Drosophila* genome.

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- ¹ Beadle, G. W., and Tatum, E. L., *Am. J. Bot.*, **32**, 678 (1945).
- ² Epstein, R. H., et al., *Cold. Spring Harb. Symp. quant. Biol.*, **28**, 375 (1963).
- ³ Hadorn, A., and Letalfaktoren, G., *Thieme Vlg. Stuttgart* (1955).
- ⁴ Grüneberg, H., *The genetics of the mouse*, 2nd ed. (Nijhoff, The Hague, 1952).
- ⁵ Garcia-Bellido, A., in *Ciba Found. Symp. 29, Cell Patterning*, 161 (Associated Scientific London, 1975).
- ⁶ Judd, B. H., Shen, M. W., and Kaufman, T. C., *Genetics*, **71**, 139 (1972).
- ⁷ Hochman, B., *Genetics*, **67**, 235 (1971).
- ⁸ Ripoll, P., and Garcia-Bellido, A., *Nature new Biol.*, **241**, 15 (1973).
- ⁹ Bryant, P., *Devl Biol.*, **22**, 389 (1970).
- ¹⁰ Garcia-Bellido, A., and Merriam, J. R., *Devl Biol.*, **24**, 61 (1971).
- ¹¹ Garcia-Bellido, A., Ripoll, P., and Morata, G., *Nature new Biol.*, **245**, 251 (1973).
- ¹² Garcia-Bellido, A., and Dapena, J., *Molec. gen. Genet.*, **128**, 117 (1974).
- ¹³ Morata, G., and Ripoll, P., *Devl Biol.*, **42**, 211 (1975).
- ¹⁴ Lindsley, D. L., and Grell, E. H., *Carnegie Instn Wash. Publ.*, No. 627 (1968).
- ¹⁵ Lewis, E. B., and Backer, F., *Drosophila Inf. Serv.*, **43**, 193 (1968).
- ¹⁶ Ferrus, A., *Genetics*, **79**, 589 (1975).

Stereotyped pattern of locomotion controlled by duration of previous tracking by a predatory insect

AMONG insects, an encounter with a particular stimulus^{1–4} or the performance of a particular activity^{5–7} may change the animal's later responsiveness to other stimuli or may prime the regular appearance of a stereotyped action pattern. Thus, various unrelated species display strictly predictable behavioural changes which depend not only on simultaneous events in the external environment as well as on general physiological variables, but also on well determined sensory or motor components of the subject's

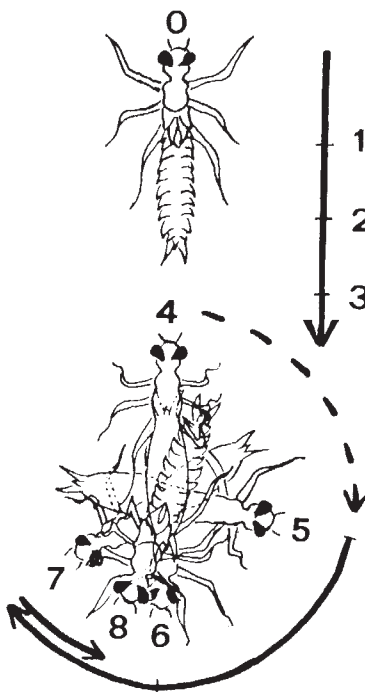


Fig. 1 The backing-turning pattern. After the disappearance of the prey stimulus, the larva (1) remains motionless (position 0); (2) it steps backwards repeatedly (solid arrow, positions 1–4); and finally (3) it turns repeatedly around its longitudinal body axis. The first pivoting movement is often accompanied by simultaneous backing (dashed arrow, position 5); thereafter, the insect turns clockwise and/or counterclockwise without changing its location (solid arrows, positions 6–8).

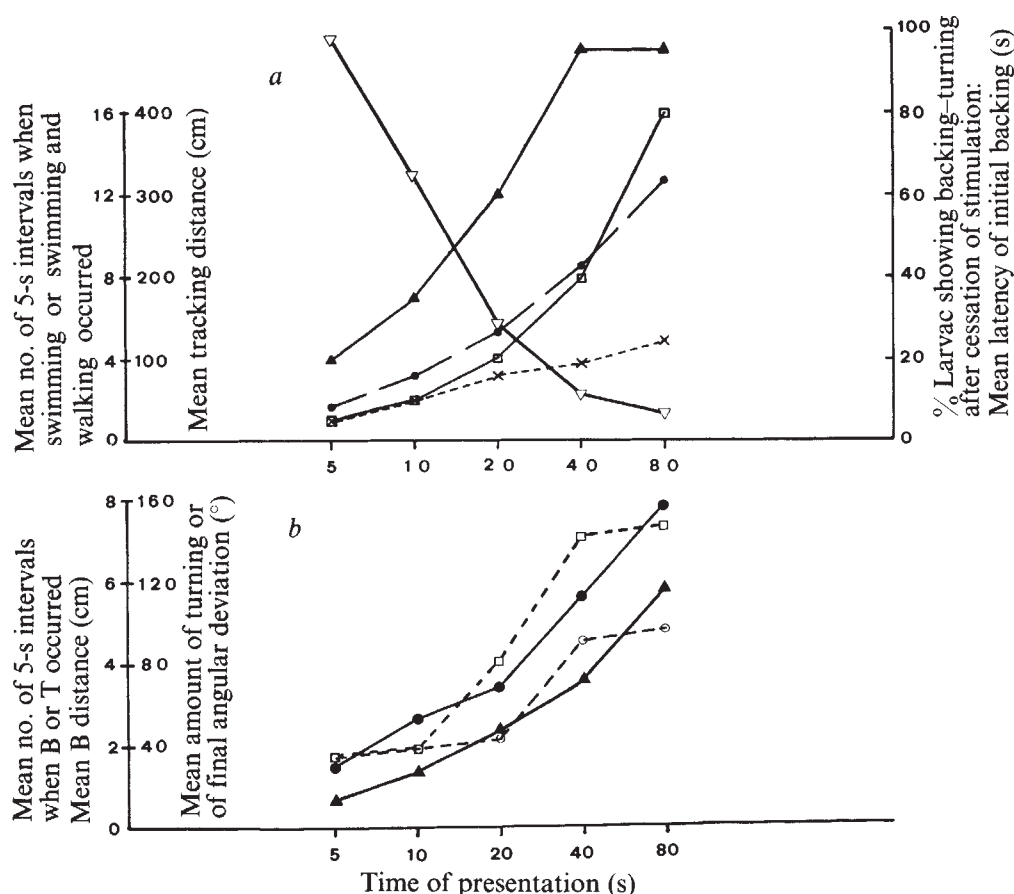


Fig. 2 *a*, Tracking time and tracking distance during presentation of the dummy for various times; percentage of larvae showing the backing-turning response within 3 min after removal of the dummy; and latency of initial backing response. B, Backing; T, turning; S, stimulation; x, swimming time; ●, tracking distance; □, total tracking time corresponding to swimming and walking; ▲, backing-turning after stimulation; ▽, latency of initial backing. The increase in the number of individuals showing the backing-turning response and the decrease in the latency of the initial backing response are significant at the 0.01 and 0.001 levels, respectively. The relationship between the time of presentation and the percentage of individuals showing backing-turning corresponds to the equation $B-T = 0.517t^{1.654}$ (where t stands for time of presentation) $n = 100$. *b*, Number of backing-turning responses, backing distance and amount of angular turning within 3 min after removal of the dummy. ●, Backing-turning response; □, turning; ▲, backing distance; ○, final angular deviation corresponding to the change in the orientation of the insect's head and body resulting from the entire sequence of backing-turning movements. Except for the final angular deviation, the changes in the values of the different backing-turning parameters are all significant at the 0.001 level $n = 60$.

antecedent behaviour. The study of these changes may aid the understanding of the nature of specific internal mechanisms which are influenced by previous behavioural states and which act in turn on the animal's subsequent behaviour. This may be of special interest in the study of highly elaborate action patterns, such as the figure-of-eight dance of the honey bee. In this example, the return of the bee from a given food source to the hive is followed by a precisely defined pattern of locomotion, in which the timing and orientation of certain parameters depend on specific aspects of the insect's outgoing flight⁸. A series of experiments analysed the reactions of the aquatic dragonfly larva *Aeschna cyanea* M. after a brief presentation of an artificial prey stimulus. They revealed that the behaviour of the insect is influenced by the nature and former location of the stimulus, even after the latter has disappeared⁹. On the other hand, after a more extended presentation of a moving target—a stimulus which typically elicits prolonged tracking behaviour—the insect reliably carries out a strongly stereotyped pattern of locomotion, the form, control and function of which are described here.

Hungry subjects of the final larval stage were placed in a large experimental aquarium (70×70 cm) and presented with a spherical dummy prey (diameter, 3.5 mm) for 5–80 s. The dummy was introduced directly into the insect's visual field and then moved around the aquarium, its velocity

being adapted to the subject's own tracking speed. At the end of the period of stimulation the dummy was removed quickly from the aquarium. The behaviour of the larvae during the presentation of the dummy as well as during the 3 preceding and 3 subsequent minutes was recorded by two cameras, one of them stationary, above the centre of the aquarium, the other fitted with a zoom lens and manipulated by the experimenter in front of the aquarium.

Presentations of the dummy during 5- and 10-s intervals elicited tracking with vigorous swimming movements; presentations of still longer duration elicited swimming movements followed by rapidly decelerating walking (and creeping) movements (Fig. 2*a*). After removal of the dummy, the insects tended to display a stereotyped pattern¹⁰ which had not been observed before or during the presentation of the dummy. It consisted of three phases: (1) the larvae remained motionless and 'stared' in the direction in which the prey stimulus had just disappeared; then they carried out (2) a series of backing movements and finally (3) a number of turning or pivoting movements around the longitudinal axis of their body, without changing their location (Fig. 1).

The longer the preceding presentation of the dummy, the sooner the 'backing-turning' pattern appeared, and the greater (1) the percentage of larvae which showed this pattern (Fig. 2*a*), (2) the number of time units in which

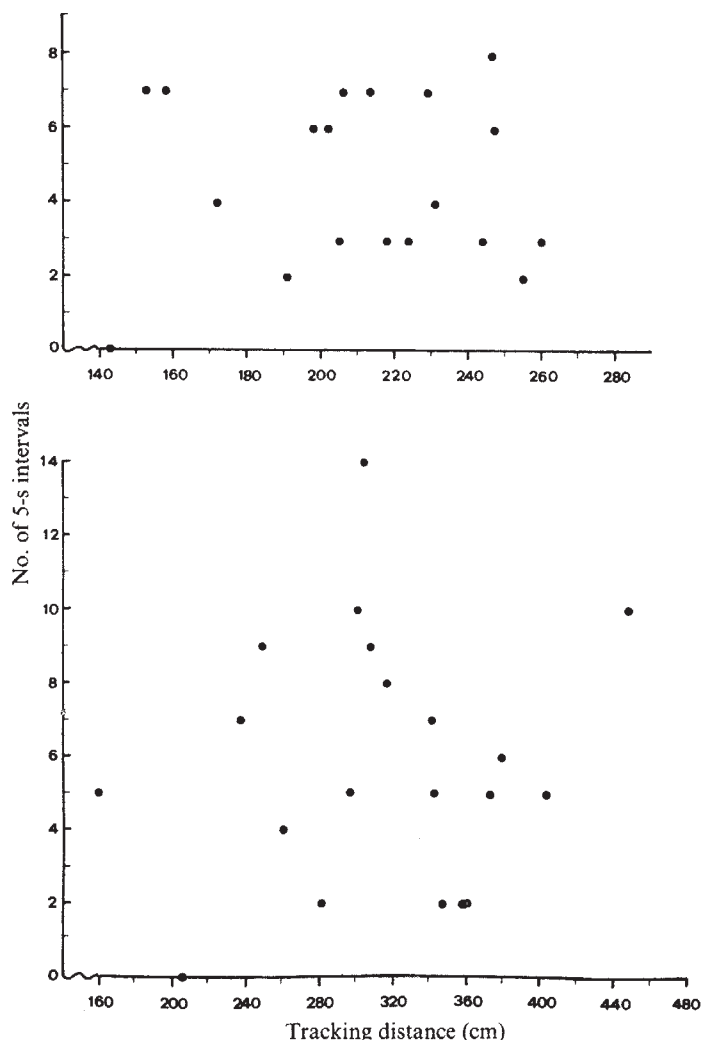


Fig. 3 Relationship between tracking distance during and number of backing-turning responses after presentation of the dummy for 40-(a) and 80-s intervals (b). The abscissa represents the tracking distance covered by individual larvae; the ordinate refers to the number of 5-s units for which a given individual displayed backing-turning responses during the first 3 min after removal of the dummy. For both experiments, the Spearman rank correlation test failed to show a significant relationship between the larvae's tracking distance and the number of backing-turning responses subsequently carried out. a, $r_s = 0.12$; b, $r_s = 0.0038$

backing and turning occurred, (3) the backing distance, (4) the amount of angular turning, and (5) the extent to which the animals had changed the orientation of their head and body as the outcome of the entire performance of the stereotyped response (either by turning repeatedly in the same direction, or by making clockwise as well as anticlockwise turning motions) (Fig. 2b).

During the two longest presentations of the target (40 and 80 s respectively), nearly all subjects followed the dummy until its removal and thus showed the same tracking time (Fig. 2a); the same individuals, however, differed considerably with respect to the distance they covered within 40- and 80-s intervals. Figure 3 shows that the correlation coefficient between the larvae's tracking distance and the number of their subsequent backing-turning responses tended towards zero. The intensity or persistence of the backing-turning response seemed therefore to depend on the time of the insects' tracking rather than on the distance they had covered.

Experiments in which the presentation of the dummy exceeded 80 s have demonstrated that after a time of presentation of 90 s or more the larvae cease to approach the prey and begin to back up and turn away from it, as if the dummy were no longer in their visual field. Thereafter, the insects might oscillate for several minutes between bouts of positive fixation, often combined with approach reactions, and short spells of negative backing-turning away from the dummy. Thus, the removal of the dummy eliminates the tracking command and therefore facilitates and synchronises the appearance of a whole sequence of backing-turning motions; it is, however, by no means a necessary condition for the release of that stereotyped pattern of locomotion.

Further experiments and observations involving the presentation of artificial as well as natural prey indicated that backing-turning occurred only during or after the presentation of a prey stimulus which had previously elicited an approach response. This stereotyped response seemed therefore to be a consequence of a specific activity, and not simply a result of the stimulus which had released this activity. Nevertheless, proprioceptive information resulting from predatory locomotion did not seem to have any influence on the occurrence of backing-turning. For instance, backing-turning movements occurred mainly after the pursuit had reached the stage of walking and creeping. Some insects, however, displayed (weak) backing-turning responses after the dummy had been presented for only a short time; in these cases, due to the brevity of presentation, the tracking responses took the form of swimming movements only. Furthermore, interpolated acts—such as striking and eating a real prey between repeated presentations of the dummy of short duration—did not reduce the cumulative after effect on backing-turning exerted by previous tracking movements. Finally, metabolic changes resulting from the energy consumption⁸ during the tracking activity likewise seemed to have no influence on the occurrence of the stereotyped pattern of locomotion. Among other things, the priming effect of tracking depended on the insects' tracking time rather than on the tracking distance, the latter parameter being correlated with the larva's energy consumption. Thus, by analogy with Blest's⁵ explanation of the priming effect which flying exerts on the stereotyped settling movements in hemileucine moths, the instigation of the backing-turning pattern may be explained by time-dependent central nervous after effects originating from the activation of those mechanisms which control tracking.

From a functional point of view one may consider that backing-turning has the following effects: (1) it stops any futile attempt on the part of the insect to catch a prey able to escape; (2) it reduces the probability that such a prey will be rediscovered after its momentary disappearance; (3) as direct observation of the larvae's hunting behaviour in the presence of living prey has shown, turning favours the detection of a new prey; (4) finally, the loss of contact with an escaping target reduces the insects' energy consumption as well as the danger that their tracking movements may attract their own enemies.

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- ¹ Dethier, V. G., *Science*, **125**, 331 (1957).
- ² Dethier, V. G., Solomon, R. L., and Turner, L. H., *J. comp. Physiol. Psychol.*, **60**, 303 (1965).
- ³ Kennedy, J. S., *J. exp. Biol.*, **45**, 215 (1966).
- ⁴ Kennedy, J. S., in *Experimental Analysis of Insect Behaviour*, 1 (edit. by Barton Browne, L.), (Springer, Berlin-Heidelberg-New York, 1974).
- ⁵ Blest, D., *Behaviour*, **16**, 188 (1960).
- ⁶ Graham, K., *Nature*, **191**, 519 (1961).
- ⁷ Bennet, R. B., and Borden, J. H., *Ann. Entom. Soc. Am.*, **64**, 1273 (1971).
- ⁸ Frisch, K. von, *The Dance Language and Orientation of Bees* (Harvard University Press, Cambridge, Massachusetts, 1967).
- ⁹ Etienne, A. S., *Anim. Behav.*, **20**, 724 (1972).
- ¹⁰ Hoppenheit, M., *Zool. Anz.*, **172**, 216 (1964).

Modulation of B-cell interactions by T cells

INTERACTION between different lymphoid and non-lymphoid cells is a well established requirement for the development of the immune response *in vivo* and *in vitro*¹⁻³. From this it follows that ultimately the regulation of the immune response must depend on the nature and frequency of such cell interactions.

Recently, in the process of examining suspensions of lymph node cells, stained with fluorescein isothiocyanate (FITC)-labelled rabbit anti-mouse immunoglobulin and obtained from mice partially depleted of T cells after treatment with anti-lymphocyte antiserum (ALS), a very high frequency of cell pairs was noticed, in contrast with control cell suspensions. We have followed up that observation and examined lymph node cell suspensions from control and ALS-treated CBA and C3H/BI mice, and mice treated with ALS and reconstituted with thymocytes, in order to analyse the frequency, characteristics and kinetics of cell pairing. We found a higher frequency of pairs in the ALS-treated mice both in the cell suspensions stained with the FITC-labelled antiserum and in unstained suspensions prepared immediately after removal of the lymph nodes, washed twice, resuspended to a concentration of 1.2×10^6 per ml and examined in a haemocytometer chamber within 30 min of dissection (Table 1).

Whether counted in slides after staining with FITC-labelled immunoglobulin or counted in haemocytometer chambers after washing, the proportions of pairs found in ALS-treated suspensions were consistently higher ($9.44\% \pm 2.02$; $6.92\% \pm 0.88$) than in the respective controls ($2.44\% \pm 1.02$; $1.31\% \pm 0.38$). Moreover, the high frequency of pairs in the ALS-treated suspensions was counteracted by reconstitution with thymocytes ($2.97\% \pm 0.34$; $3.25\% \pm 1.26$).

In addition to the quantitative differences between the cell suspensions, qualitatively, the nature of the cell pairs examined also differed (Table 2). After ALS treatment, the increase in the numbers of pairs was due chiefly to homologous B-cell pairs ($4.47\% \pm 0.95$) and heterologous pairs ($3.74\% \pm 1.83$). Only a small proportion of pairs ($1.07\% \pm 0.90$) consisted of two unlabelled lymphoid cells, presumably T cells. Larger aggregates of cells, consisting chiefly of B cells, were also present in the smears of the ALS-treated lymph node cell suspensions, examination of these under phase contrast, revealed that, although some of the unlabelled cells were clearly lymphoid, some heterologous pairs consisted of one macrophage and one B cell. In the control cell suspensions most of the few existing

Table 1 Percentage of cell pairs in lymph node suspensions of ALS-treated and control mice, and ALS-treated mice reconstituted with thymocytes

Treatment	No. of mice	Slide	Pairs (%) Haemocytometer
—	6	2.44 ± 0.83	1.31 ± 0.38
ALS*	12	9.44 ± 2.02	6.92 ± 0.88
ALS $\pm$ thymocytes†	4	2.97 ± 0.34	3.25 ± 1.26

Cells were examined unstained (haemocytometer) or after staining with an FITC-labelled antiserum (slide). The ALS-treated mice received 1.5 ml of ALS (Searle) at 2–3 months old, distributed in 3 subcutaneous injections of 0.5 ml given on alternate days before killing. This induced moderate decreases in the proportion of T cells (untreated: 72.8 ± 8.14 ; ALS: 54.3 ± 11.5) in the mesenteric and inguinal lymph node suspensions.

ALS-treated thymocyte-reconstituted mice received 1 ml or 0.5 ml ALS on day 0, on day 1 they received an intraperitoneal injection of 3.6×10^7 syngeneic thymocytes; and they were killed on day 2.

Cells examined in the haemocytometer were adjusted to 1×10^6 ml⁻¹ in MEM or medium 199.

Cells examined in microscope slides were stained with rabbit anti-mouse immunoglobulin and counted by the standard direct immunofluorescence technique. Minimum of 300 cells counted.

Means $\pm$ s.d.

* $P < 0.00001$.

† $P < 0.5$.

Table 2 Nature of cell pairs found in lymph node suspensions* of ALS-treated and control mice, and ALS-treated mice reconstituted with thymocytes

Group	Pairs (%)	-/-	-/+	+/+
Control	2.44 ± 1.02	1.71 ± 0.81	0.3 ± 0.35	0.57 ± 0.48
ALS	9.44 ± 2.02	1.07 ± 0.90 ($P < 0.25$)	3.74 ± 1.83 ($P < 0.005$)	4.74 ± 0.95 ($P < 0.00001$)
ALS + thymocytes	2.97 ± 0.34	1.33 ± 0.84 ($P < 0.61$)	0.94 ± 0.04 ($P < 0.07$)	0.71 ± 0.54 ($P < 0.77$)

*Stained with an FITC-labelled rabbit anti-mouse immunoglobulin antiserum. See legend to Table 1. -/-: Homologous, surface IgG negative; -/+ : heterologous, consisting of 1 labelled and 1 unlabelled cell; +/+ : homologous, surface IgG positive.

Mean $\pm$ s.d.

pairs consisted of two unlabelled lymphoid cells (1.71 ± 0.81). Reconstitution with thymocytes resulted in a low frequency of pairs, comparable with that observed in the controls, that is, most of the existing pairs (1.33 ± 0.84) consisted of two unlabelled lymphoid cells.

Finally the kinetics of pair formation in the control and ALS-treated populations was studied in Couette viscometer chambers where cells are allowed to collide in well controlled conditions of constant shear rate, viscosity and temperature (Table 3). Although some variation was observed between control and experimental groups, in individual experiments the ratios between the ALS-treated and control suspensions within each experiment were similar. The numbers of pairs in the partially T-cell depleted suspensions were always higher than in the controls (reflected by ratios > 1); at 7 min the pairs in both suspensions tended to disaggregate (reflected in the ratio nearest 1, 1.43) but at the later times, 21 and 28 min, re-aggregation was observed at higher levels in the partially T-cell depleted suspensions. In conclusion, the present results indicate that partial T-cell depletion leads to an increase in the frequency of heterologous and B-B direct cell interactions, which can be reversed by reconstitution with thymocytes. It has been shown previously that both T and B lymphocytes release factors capable of diminishing the adhesion of the unlike cell type (B or T) *in vitro*⁵. This study is the first definitive indication that such factors must be operating *in vivo*. The increase in the numbers of direct B-cell interactions must occur as the consequence of a decrease in the concentration of T factor; whether the partial reduction in the numbers of T cells is sufficient to lead to that decrease or whether ALS has an additional inhibitory action on the release of the T-cell factor needs to be clarified. Whatever the exact mechanism, the effect was reversed by the *in vivo* administration of thymocytes.

The relevance of these observations to current theories of the mechanism of T-cell regulation of the immune response is obvious. They clearly offer an attractive cellular explanation of the finding of enhanced antibody production in partially T-cell depleted animals⁶ (reviewed in ref. 7). If, in a partially T-cell depleted population of lymph node cells obtained in the absence of a deliberately administered antigen the chance of direct interactions between different cell classes increases so considerably, addition of antigens, especially thymus independ-

Table 3 Kinetics of cell aggregation

Time (min)	Ratio* (ALS treated/control)
0	3.31 ± 1.58
7	1.43 ± 1.00
14	2.04 ± 1.08
21	2.11 ± 0.73
28	2.17 ± 1.10

*Mean $\pm$ s.d. (5 experiments).

Aggregation was measured in Couette viscometers⁴ set at shear rates of 10 s^{-1} , at 37°C .

ent, will meet with the most favourable physical conditions for specific information to be passed economically between interacting cells. The requirement of cell aggregation for immunostimulation to occur has been demonstrated in other *in vitro* studies of cell interaction⁸. Moreover, we have now shown that administration of thymocytes reduces the number of B-B cell interactions. The most likely functional repercussion of such an effect *in vivo* would be suppressed or defective antibody production as a direct result of diminished B-cell activation. The suppressive effect of thymocytes administered to mice immunised with SSSIII has been elegantly demonstrated by the extensive work of Baker and his associates⁹. The question of how T cells exert a regulatory role of B-cell function has been the subject of much discussion. The mechanism we have now been able to visualise, strikes us as a simple and ingenious control device which could be operating in most experimental systems *in vivo* and *in vitro* in which helper versus suppressive effects have been described¹⁰.

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- ¹ *Transplant. Rev.*, **1**, (1969).
- ² Mitchison, N. A., Rajewsky, K., and Taylor, R. B., in *Developmental Aspects of Antibody Formation and Structure*, 2 (edit. by Sterzl, J., and Riha, I.), 547 (Academic, New York, 1971).
- ³ Katz, D., and Benacerraf, B., *Adv. Immun.*, **15**, 2 (1972).
- ⁴ Curtis, A. S. G., *J. Embryol. exp. Morph.*, **22**, 305 (1970).
- ⁵ Curtis, A. S. G. and de Sousa, M., *Cell. Immun.*, **19**, 282 (1975).
- ⁶ Anderson, H. R., et al., *Immunology*, **22**, 277 (1972).
- ⁷ *Transplant. Rev.*, **26** (1975).
- ⁸ Scheerer, W. T., and Parker, C. W., *J. Immun.*, **115**, 613 (1975).
- ⁹ Baker, P., *Transplant. Rev.*, **26**, (1975).
- ¹⁰ Katz, D. H., *Transplant. Rev.*, **12**, 141 (1972).

Effect of interferon on lymphocyte transformation and nuclear antigen production by Epstein-Barr virus

THE Epstein-Barr virus (EBV) can transform human and simian lymphocytes into blast cells which are then capable of indefinite growth *in vitro*¹⁻⁴, with 85-100% of the cell population expressing the EBV-induced nuclear antigen (EBNA). Biological⁵⁻⁷ and molecular⁸ evidence shows clearly that there are at least two types of EBV strains, one of which (P3HR-1 EBV) does not transform human lymphocytes^{5,6} and seems to possess approximately 15% more DNA sequences than the highly transforming strain (B95-8 EBV)⁸. Furthermore, by infecting cells of the established EBV genome-negative BJA-B line⁹ it was also possible to show that whereas B95-8 EBV induces only EBNA in these cells, P3HR-1 EBV induces both EBNA and early antigen (EA)⁷. Thus, by using cells of BJA-B line, it now seems possible to investigate further several known viral functions¹⁰. We report here the results of a study in which we found that lymphocyte transformation by B95-8 strain of EBV could not be prevented by interferon. As shown below, this finding is further supported by the observation that in spite of the presence of interferon in very high doses (that is, up to 10^4 IU ml⁻¹), EBNA can still be produced in a fraction of the EBV-infected BJA-B cells.

It is already known that interferon may not only inhibit virus replication but also cell transformation by viruses¹¹⁻¹⁴. Herpes viruses in general, however, seem to be relatively insensitive to the antiviral effect of interferon (see ref. 15 for review). The effect of interferon on cell transformation by EBV and other herpesviruses has not yet been described. Moreover, the effect of interferon on the expression of EBNA has not been investigated.

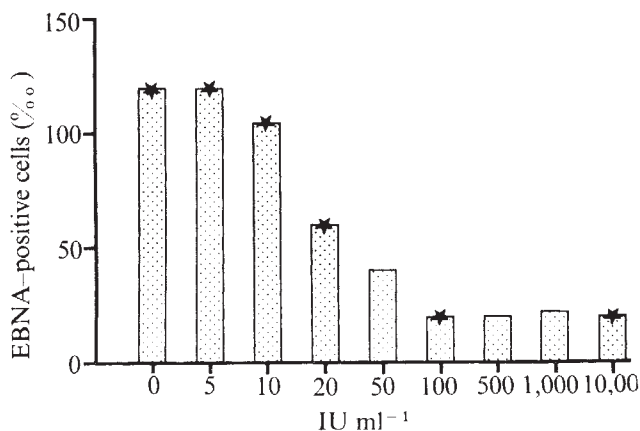


Fig. 1 Effect of interferon on EBNA production in BJA-B cells infected with B95-8 strain of EBV. Interferon was added to the culture medium 24 h before EBV infection. Interferon at these same concentrations was also added to the new medium used after infection, and the cells incubated for 72 h at 37°C. Smears were prepared as described in Table 1. Whereas the results at 50, 500 and 1,000 IU are from single experiments, all others represent means from two separate experiments.

The experimental approach and the data on the effect of interferon on lymphocyte transformation are given in Table 1. Pretreatment of cells for 24-72 h with the interferon even at doses as high as 10^4 IU ml⁻¹ did not inhibit lymphocyte transformation by EBV. No significant differences were observed in terms of time required for lymphocyte transformation by EBV (that is, development of a lymphoblastoid cell line: LCL) irrespective of the various interferon concentrations used. 85-97% cells from the transformed cultures contained EBNA when tested 3 weeks after infection. It is now well known that the presence of EBNA in the cells of a continuously growing lymphocyte culture is the best available evidence for EBV-induced transformation^{5,10}. Several attempts to try to quantitate any effect of interferon on lymphocyte transformation by EBV were unsuccessful, since plating of EBV-infected CBL gave negative results in our hands (that is, EBV-infected CBL did not generally grow or stay viable for more than 5-7 d in semi-solid media, even in the presence of conditioning medium). We then decided to determine if CBL were sensitive to the anti-viral effect of interferon. Thus, using herpes simplex viruses we found that interferon can protect a large fraction of the cells against the cytotoxic effect of these viruses (Table 2).

Fig. 2 Effect of interferon (●) on the kinetics of EBNA expression in EBV-infected BJA-B cells. ○, EBV alone. Cells were first cultured for 24 h in medium containing interferon (100 IU ml⁻¹) and infected as described in Table 1. The new culture medium added after EBV infection also contained interferon at this same concentration. Results represent means of two separate experiments.

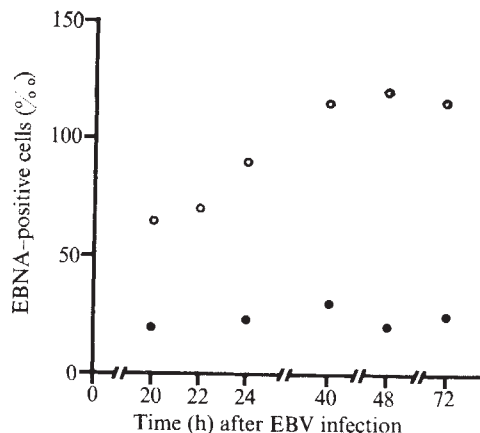


Table 1 Transformation of CBL by the B95-8 strain of EBV in the presence of interferon*

	24, 48 and 72 h before infection	Interferon added simultaneously with EBV	12, 24 and 48 h after infection	No interferon
Time (d) required for transformation† % EBNA-positive cells at 4 weeks	10-16 > 85	10-16 > 85	10-16 > 85	10-16 > 85

Lymphocyte transformation assays were carried out using human umbilical cord blood lymphocytes (CBL). CBL separation, culture, virus preparation and titration, EA and VCA (virus capsid antigen) staining procedures as well as tests for EBV specificity, were as described previously⁵. Two different preparations of human interferon, one concentrated and the other partially purified, containing 7.5×10^5 interferon units (IU) ml^{-1} (10^4 IU per mg protein) and 2.5×10^6 IU ml^{-1} (10^5 IU per mg protein) respectively, produced in Sendai virus-treated leukocytes, were supplied by Dr Kari Cantell (Central Public Health Laboratory, Helsinki). The procedures for interferon preparation, concentration and titration were as described previously¹⁶. The anti-viral activity of the interferon preparations was confirmed in our laboratory by a microassay system¹⁷ on human embryo fibroblasts using Sindbis virus as challenger.

For EBNA induction in BJA-B, 10^6 cells were pelleted by a low speed centrifugation and the pellet resuspended in 0.5 ml of a virus dilution (known to be capable of inducing EBNA in about 12% of cells, collected 48 h after EBV infection). The cells were then gently dispersed and the mixture incubated for 1 h at 37°C in a CO_2 -humidified incubator. Growth medium (RPMI 1640 containing 10% foetal bovine serum and antibiotics)⁹ was then added to bring the cell concentration to 10^6 cells ml^{-1} and this suspension was dispersed in culture tubes which were then kept at 37°C for a desired period of incubation. The anti-complement immunofluorescence method¹⁸ was used for EBNA staining but with the following modifications. JM reference serum¹⁸ with anti-EBNA + VCA + EA antibody composition, diluted 1:5 in phosphate-buffered saline (PBS, pH 7.2) was used as common source of anti-EBNA antibody and complement; fresh JM serum in 0.2 ml aliquots was kept frozen at -90°C until use. Smears with about 10^5 cells per coverslip were air dried at room temperature and fixed for 3 min in a 1:1 mixture of chilled (-20°C) acetone-methanol and kept frozen at -20°C until use. For EBNA reaction, fixed smears were incubated at room temperature for 45 min with fresh JM serum diluted to 1:5 in PBS, then washed twice with the latter and incubated for 45 min at room temperature with anti-human B₁C/B₁A FITC conjugate (Hyland Laboratories) at 1:20 dilution. The smears were subsequently washed (in PBS) three times, counterstained with Evans blue, washed three times and mounted on a slide in a 1:1 mixture of glycerol-PBS. Controls included (a) uninfected BJA-B cells, (b) EBV genome-positive Raji cells, (c) heat-inactivated (56°C per 30 min) JM serum and (d) fresh EBV-0 serum, without detectable anti-EBV antibody, derived from a healthy EBV-seronegative donor.

*First, CBL of four different donors were used for these experiments. Interferon concentrations tested were 10^2 , 10^3 and 10^4 IU ml^{-1} , respectively, and maintained by adding interferon to the new medium at every change. The EBV preparation used here had a transforming titre of $10^{5.5}$ log₁₀TDC₅₀ ml^{-1} . Infection was carried with 0.1 ml virus suspension per 10^6 CBL. The experiments were then repeated with CBL of two other donors with a new, partially purified interferon preparation (see above). At least two cultures (tubes and flasks) per donor were used in each of the above experiments.

†Transformation was confirmed by the criteria defined previously⁵. No transformation was observed in uninfected cultures, and the transforming activity was neutralised by pretreating the virus suspension with reference JM serum containing anti-EBV antibody. Serum from EBV-seronegative donor did not neutralise this transforming activity.

In preliminary experiments using BJA-B cells and CBL, we had found that in both cell types interferon had a similar inhibitory effect on the induction of EBNA by EBV. We therefore decided to quantify this effect only in the EBV genome negative BJA-B cell line. In BJA-B cells, the expression of EBNA could not be completely inhibited by interferon, even at doses as high as 10^4 U ml^{-1} . There was no detectable interferon effect (reduction in number of EBNA-positive cells) at 5 IU ml^{-1} but a considerable reduction was observed at doses over 10 IU ml^{-1} . Doses of 10^2 – 10^4 IU ml^{-1} gave similar results (Fig. 1). In preliminary experiments during the present study we found that in our experimental conditions the number of EBNA-positive BJA-B cells reached its maximum 40–48 h following EBV infection. Therefore, we investigated the effect of interferon on the appearance of EBNA up to 76 h after EBV infection. Figure 2 shows that a considerable reduction in the number of EBNA-positive cells was consistently detected in the presence of interferon in all the samples collected 20–72 h after EBV infection. Two other single experiments carried out using a more concentrated virus preparation (capable of inducing EBNA in about 20% of BJA-B cells), as well as interferon treatment of the cells beginning 1 week before EBV infection, also gave similar results. From these findings, it is difficult to understand why the anti-viral (EBNA) effect of interferon is effective only in a fraction of BJA-B cells. It is, however, possible that a subpopulation of these cells is interferon resistant. In EBV-infected CBL cultures such a resistant cell population would outgrow the protected cells during the period (10–16 d) up to when the transformation is confirmed, and would thus explain the observed interferon resistance of CBL transformation by EBV. Note, however, that in other virus-cell systems interferon resistance in a fraction of virus populations¹⁹ and/or a fraction of virus-infected cells²⁰ have been considered in the past as possible explanations for such a phenomenon.

Interestingly, it was previously found that interferon was unable to block the enhanced expression of EBV-induced

Table 2 Sensitivity of CBL to the anti-viral action of interferon as tested against cytocidal effect by herpes simplex viruses types 1 and 2*

Virus type	Virus dose (log ₁₀ TCD ₅₀ ml^{-1})	Interferon concentrations (U per 0.2 ml per 200,000 cells)			
		0	10	100	1,000
HSV 1	10^2	12†	38	70	71
	10^3	10	20	30	39
HSV 2	10^2	24	20	65	63
	10^3	10	17	40	42
Control (CBL cultures)	—	92	99	99	97

*CBL from a single donor were preincubated with interferon for 48 h. Virus was then added to these cultures which were further incubated for 96 h at 37°C , and the cells examined for viability by Trypan blue dye exclusion test.

†Figures represent percentage of viable cells in each of the above conditions.

antigens by hydrocortisone in EBV genome-carrying lymphoblastoid cell lines²¹. Note, however, that interferon has an inhibitory effect on cell transformation by other well known DNA viruses such as SV40 and adenoviruses^{12–14}; this effect may, however, vary in degree according to the cell system and the virus used^{12,13}. On the other hand, the effect of high interferon concentrations (10^4 IU ml^{-1}) on cell transformation as used in the present study was not tested against SV40 and adenoviruses^{12,13}. Interestingly, the effect of interferon on EBNA production seems to be similar to its effect on T-antigen synthesis by SV40 and adenoviruses. In this respect, it was suggested that the expression of SV40 genome is sensitive to the action of interferon only if the SV40 genome is not integrated into the cell genome^{13,22}. Since the question of the integration of the EBV genome into the cellular genome has not yet been completely resolved, it may be very important to transform CBL in the presence and/or absence of interferon and examine the LCL thus obtained for the relation of EBV genome to cellular genome in terms of integration.

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- 1 Henle, W., Diehl, V., Kohn, G., Zur Hausen, H., and Henle, G., *Science*, **157**, 1064-1065 (1967).
- 2 Pope, J. H., Horne, M. K., and Scott, W., *Int. J. Cancer*, **3**, 857-866 (1968).
- 3 Gerber, P., Whang-Peng, J., and Monroe, J. H., *Proc. natn. Acad. Sci. U.S.A.*, **63**, 740-747 (1969).
- 4 Miller, G., Shope, T., Lisco, H., Stit, D., and Lipman, M., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 383-387 (1972).
- 5 Menezes, J., Leibold, W., and Klein, G., *Expl Cell Res.*, **92**, 478-484 (1975).
- 6 Miller, G., Robinson, J., Heston, L., and Lipman, M., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4006-4010 (1974).
- 7 Klein, G., Sudgen, B., Leibold, W., and Menezes, J., *J. Intervirology*, **3**, 232-244 (1974).
- 8 Pritchett, R. F., Hayward, S. D., and Kieff, E. D., *J. Virol.*, **15**, 556-569 (1975).
- 9 Menezes, J., Leibold, W., Klein, G., and Clements, G., *Biomedicine*, **22**, 276-284 (1975).
- 10 Menezes, J., Leibold, W., and Klein, G., in *Oncogenesis and Herpesviruses* (International Agency for Research in Cancer, Lyon, II (edit. by de Thé, G., Epstein, M. A., and zur Hausen, H.), 323-330 (1975).
- 11 Isaacks, A., *Adv. Virus Res.*, **10**, 1-38 (1963).
- 12 Todaro, G. J., and Baron, S., *Proc. natn. Acad. Sci. U.S.A.*, **54**, 752-756 (1965).
- 13 Oxman, M. N., Rowe, W. P., and Black, P. H., *Proc. natn. Acad. Sci. U.S.A.*, **57**, 941-948 (1967).
- 14 Oxman, M. N., in *Interferons and Interferon Inducers* (edit. by Finter, N. B.), 395-480 (North Holland, Amsterdam, 1973).
- 15 Lockart, R. Z., in *The Herpesviruses* (edit. by Kaplan, A.), 262-269 (Academic, New York, 1973).
- 16 Cantell, K., Kirvonen, S., Mogensen, K. E., and Pyhälä, L., in *The Production and use of Interferon for the Treatment and Prevention of Human Virus Infections*, *In Vitro*, Monograph No. 3, 35-38 (1974).
- 17 Dahl, H., and Degre, M., *Acta path. microbiol. scand.*, **B80**, 863-870 (1972).
- 18 Reedman, B. M., and Klein, G., *Int. J. Cancer*, **11**, 499-520 (1973).
- 19 Takemoto, K. K., and Baron, S., *Proc. Soc. exp. Biol. Med.*, **121**, 670-675 (1966).
- 20 Oie, H. K., Easton, J. M., Ablashi, D. V., and Baron, S., *Infect. Immun.*, **12**, 1012-1017 (1975).
- 21 Joncas, J., Bouchard, J., Boudreault, A., and Granger-Julien, M., *Cancer Res.*, **33**, 2142-2148 (1973).
- 22 Oxman, M. N., *et al.*, *Virology*, **32**, 122-127 (1967).

Growth of bacilli on methyl- α -D-glucoside

METHYL- α -D-GLUCOSIDE (α MG) is an analogue of glucose that is used extensively for studies of glucose transport in microorganisms^{1,2}. This use implies that the analogue enters the cell and is phosphorylated², but is not further metabolised. It is known² that *Escherichia coli* do not grow on α MG and Freese *et al.*³ reported that *Bacillus subtilis* (strain 60015) can grow on α MG only after a long lag

period: the subsequent growth rate being very slow. This may not, however, be typical of bacilli since here we report several strains of bacilli which grow readily on α MG.

E. coli (KL16), *B. stearothermophilus* var. *non-diastaticus*, *B. subtilis* (Marbourg) and *B. licheniformis* (A5) were grown on nutrient broth and then inoculated into salts medium (for *E. coli* see ref. 4; for *B. stearothermophilus* ref. 5; for the others ref. 6) containing 25 mM of one of the following sugars as the sole carbon source: α MG, 2-deoxyglucose, cellobiose, melibiose, maltose, lactose, sucrose or glucose. The cultures were incubated aerobically overnight (*B. stearothermophilus* at 55 °C, the others at 37 °C) to establish which sugars supported the growth of these organisms. The results show

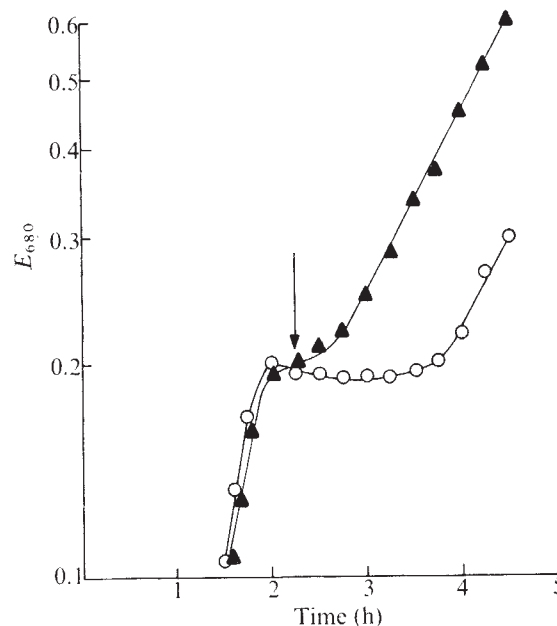


Fig. 1 Growth of *B. licheniformis* on glucose (○), and maltose (▲), and subsequent growth on α MG (2 mM added as indicated by the arrow).

that whereas *E. coli* does not grow on α MG (nor sucrose, nor cellobiose), all of the bacilli tested grew readily on these carbohydrates. All organisms tested grew readily on glucose and maltose, but not on 2-deoxyglucose.

In view of the fact that the bacilli grow on α MG, the growth rates and yield of cells were compared from bacilli growing on α MG and on glucose. *B. stearothermophilus*, *B. licheniformis*, *B. megaterium* and *B. subtilis* were grown on nutrient broth and then inoculated into salts medium containing either 2 mM glucose or 2 mM α MG. The cultures were incubated aerobically at 37 °C and growth followed by measuring the absorbance of the cultures at 680 nm. When both of the cultures had just run out of substrate, α MG to 2 mM was added to the culture that had grown on glucose and the time before growth of the culture began on the new substrate was recorded as the lag period for the organism to adapt from metabolising

Table 1 Growth of *B. stearothermophilus*, *B. licheniformis*, *B. megaterium* and *B. subtilis* on glucose and α MG

Organism	Incubation temperature (°C)	Glucose (2 mM)			α MG (2 mM)		Lag period (min)
		Doubling time (min)	ΔE_{680}		Doubling time (min)	ΔE_{680}	
<i>B. licheniformis</i>	37	50	0.315		90	0.405	120
<i>B. megaterium</i>	37	45	0.420		70	0.520	90
<i>B. subtilis</i>	37	60	0.390		120	0.460	50
<i>B. stearothermophilus</i>	55	50	0.285		70	0.365	75

The organisms were first grown on nutrient broth and then inoculated into salts medium containing 2 mM of either glucose or α MG. When the glucose cultures had exhausted the added glucose, 2 mM α MG was added. The interval before exponential growth resumed was recorded as the lag period between the metabolism of glucose and that of α MG.

glucose to metabolising α MG. The results (Table 1) show that all the bacilli grow more slowly on α MG than on glucose but that the yield of cells from each substrate is about the same. Furthermore, there is a lag period between a bacillus metabolising α MG and metabolising glucose. In contrast, bacilli grown on maltose will immediately grow on α MG with no corresponding lag (Fig. 1). This suggests that bacilli possess a methyl- α -glucosidase which could be similar to that demonstrated in yeast (*S. cerevisiae*) which was shown to be specific for α MG in this organisms, and was coincided by growth on maltose⁷⁻⁹.

The results of this study show that bacilli grow readily on α MG, the metabolic enzyme (possibly methyl- α -glucosidase similar to that in yeast) being induced by growth on maltose and α MG. This means that α MG can only be used as a non-metabolisable analogue of glucose in bacilli if the organism has not been grown on maltose, and providing α MG is used for only a short period of time.

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- ¹ Kaback, H. R., *A. Rev. Biochem.*, **4**, 561 (1970).
- ² Lin, E. C. C., *A. Rev. Genet.*, **4**, 225 (1970).
- ³ Freese, E., Klofat, W., and Galliers, E., *Biochim. biophys. Acta*, **222**, 265 (1970).
- ⁴ Ashworth, J. M., and Kornberg, H. L., *Proc. R. Soc.*, **B165**, 179 (1966).
- ⁵ Sundaram, T. K., Cazzulo, J. J., and Kornberg, H. L., *Biochim. biophys. Acta*, **192**, 355 (1969).
- ⁶ Gary, N. D., and Bard, R. C., *J. Bact.*, **64**, 501 (1952).
- ⁷ Spiegelman, S., Sussman, M., and Taylor, B., *Fedn Proc.*, **9**, 120 (1950).
- ⁸ Khan, N. A., and Eaton, N. R., *Biochim. biophys. Acta*, **146**, 173 (1967).
- ⁹ van Stevenick, J., *Biochim. biophys. Acta*, **203**, 376 (1970).

Methyl- α -D-glucoside uptake and splitting by a thermophilic bacillus

THE presence and properties of a phosphoenolpyruvate (PEP)-dependent phosphotransferase system in a thermophilic bacillus has been described recently by Harris and Kornberg. The formation of the glucose and methyl- α -D-glucopyranoside (α MG) uptake and phosphorylation is induced by glucose¹.

In the course of a study on the transport of glucose and α MG in the same thermophilic bacillus² we noticed that growth occurred in a minimal medium supplemented with α MG as the sole carbon source. The generation time at 58 °C was found to be 52 min. The growth rates in presence of α MG, glucose and succinate are given in Fig. 1. In addition to α MG, other α -glucosides, that is, maltose, sucrose, trehalose and turanose can also serve as sole carbon sources.

Washed suspensions of α MG grown cells incorporated ¹⁴C- α MG into the macromolecular constituents of the cell (Table 1). Almost no ¹⁴C- α MG was incorporated with suspensions of glucose-grown cells. Cellular extracts of α MG and maltose-grown cultures which were allowed to take up ¹⁴C- α MG showed by thin-layer chromatography and autoradiography several spots, representing, presumably, different degradation products of the sugar; whereas extracts from cells grown on glucose revealed only a single spot in addition to the residual α MG (Fig. 2), corresponding to an α MG phosphate (to be published).

The above results demonstrate the presence of an enzyme system in the thermophilic bacillus responsible for the hydrolysis (and metabolism) of α MG. A study was carried out to identify the enzyme(s) and conditions for their

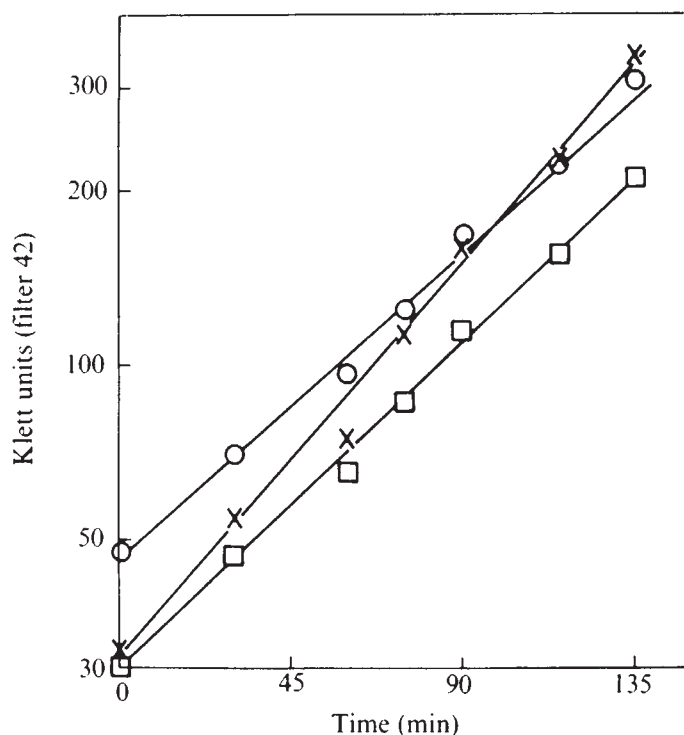


Fig. 1 Growth of the thermophilic bacillus at 58 °C on various carbon sources; 0.5% α MG (○), 0.5% Na-succinate (□) and 0.5% glucose (×). The tyrosine-leucine auxotroph and the TL-minimal medium were used; growth conditions were as described elsewhere³.

activity, using intact cell suspensions and *p*-nitrophenyl- α -D-glucopyranoside (PNPG) as substrate. PNPG hydrolysis was linear with time for about 30 min and cell concentrations up to at least 0.25 mg dry weight.

High α MG-hydrolysing activity was induced by α MG or maltose; turanose and trehalose were much poorer inducers, while sucrose was completely inactive (Fig. 3). Higher levels of induction were obtained in rich medium than in the minimal medium.

In contrast to the ability of induced intact cells to split PNPG or α MG, no activity was found when using cell-free preparations. Moreover, impairment of the integrity of the cell membrane (by treatment with toluene, sonic oscillation or lysozyme) resulted in the loss of the α -glucosidase activity. Addition of phosphoenolpyruvate (PEP), glucose-6-phosphate or adenosine triphosphate (10 μ mol each) to the cell-free system failed to restore enzymatic activity.

It was shown by Harris and Kornberg¹ that glucose induces the uptake and phosphorylation of glucose and α MG by cultures of the thermophilic bacillus grown in either acetate or succinate. As shown in Table 2 the PEP-dependent phosphorylation of ¹⁴C- α MG was induced not

Table 1 ¹⁴C- α MG incorporation into the macromolecular fraction of the thermophilic bacillus

Carbon source for growth	c.p.m. per sample	
	Whole cells	5% TCA precipitate
Na succinate + glucose	4,700	360
Na succinate + α MG	21,045	11,680
α MG	22,076	12,420

Cells were grown at 58 °C in TL-minimal medium³ supplemented with the indicated carbon source. Duration of uptake of ¹⁴C- α MG (1×10^{-4} M; specific activity, 3 μ Ci μ mol⁻¹), in medium lacking carbon source, was 10 min. Thereafter, two 0.2-ml samples, 0.1 mg dry weight of cells each, were taken for determinations of the total uptake and radioactivity in cold TCA (5%) precipitate³. Uptake refers to the radioactivity accumulated in cells, and thus includes both label of the sugar and metabolic products derived from it.

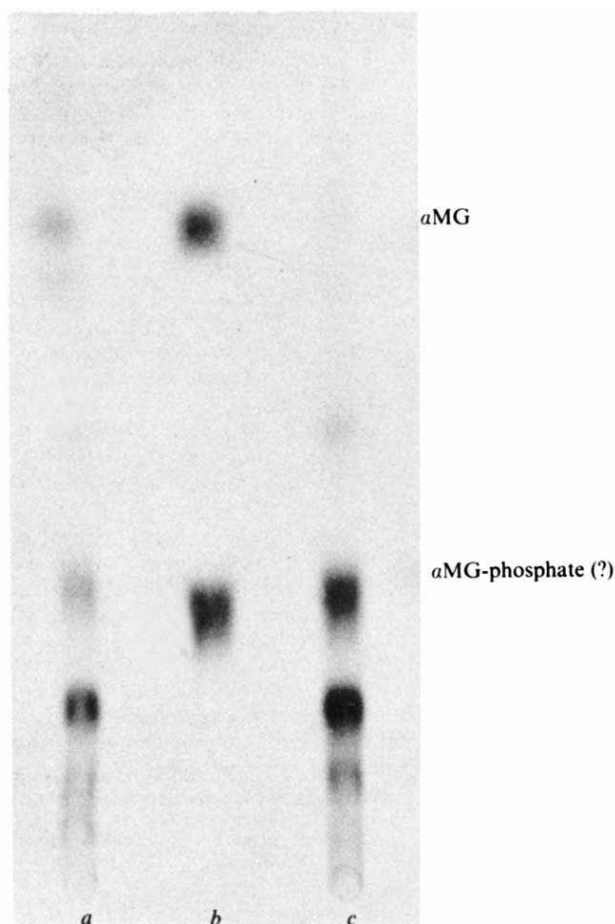


Fig. 2 Metabolism of ^{14}C - αMG . ^{14}C - αMG (2×10^{-4} M, specific activity $3 \mu\text{Ci } \mu\text{mol}^{-1}$) was added to washed suspensions (0.3 mg dry weight per ml) from *a*, αMG ; *b*, glucose and *c*, maltose grown cultures; total volume 4 ml. After 1 min at 58°C the cells were collected on membrane filters, washed and immediately plunged into boiling 1 mM Tris-HCl buffer at pH 9.0 and held for 6 min. Cell debris was removed by centrifugation. The supernatant fluid was concentrated by lyophilisation and aliquots were chromatographed on cellulose-coated sheets using ammonium acetate 1 M (pH 4.0)-95% ethanol (2:5 v/v) solvent system. The autoradiograms were observed after 7 d of exposure.

only with glucose, but also with αMG . According to these investigators¹ the uptake of αMG by intact cells of the thermophilic bacillus was directly correlated with the activity of the phosphotransferase, which catalyses the PEP-dependent phosphorylation of αMG . It seemed, therefore, worthwhile to test the possibility that αMG induces its own transport. Figure 4 shows that induction of αMG transport could indeed be achieved not only by glucose but also by αMG ; the differential rate of the synthesis of the

Table 2 Induction of αMG phosphotransferase activity

Incubation mixture	Cell-free extract from cultures grown on		
	Succinate	Succinate + glucose	Succinate + αMG
Complete	1,500	10,400	6,170
Without PEP	910	1,015	330
Without PEP; + ATP	1,020	2,030	505

Cultures were grown in TL-minimal medium with the indicated carbon sources and were collected in the log phase; the cells were washed and resuspended in 0.05 M potassium phosphate buffer (pH 7.2), treated with lysozyme (0.1 mg ml^{-1}) and then sonicated for 60 s using the MSE sonifier. The sonicates were centrifuged for 10 min at 750 r.p.m. and the supernatant assayed for ^{14}C - αMG phosphorylation ability at 58°C according to Freeze *et al.*⁴. The reaction was stopped after 10 min and assayed for DEAE-cellulose-adsorbable radioactivity. Results are expressed in c.p.m. per mg protein.

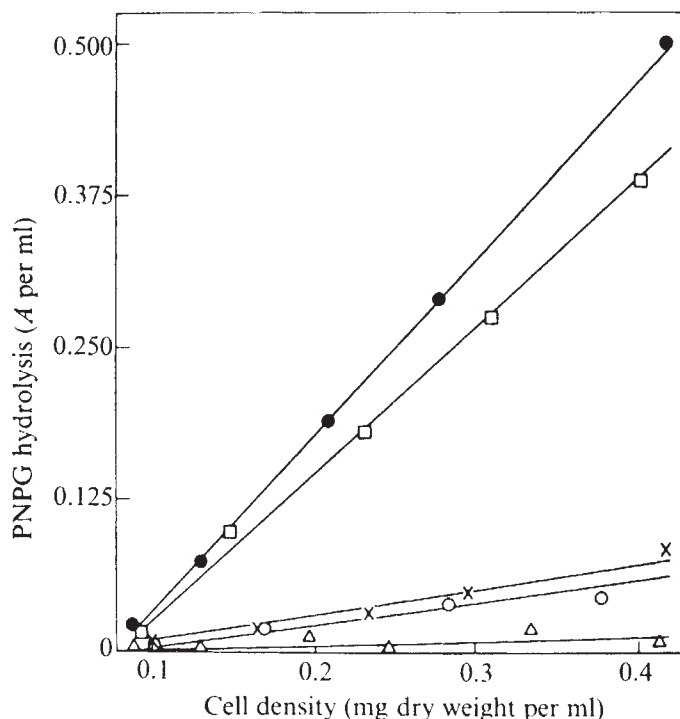


Fig. 3 Induction pattern of PNPG splitting activity by cell suspensions. Cultures were grown at 58°C in TL-minimal medium supplemented with 10% (v/v) rich medium³. For PNPG hydrolysis samples were withdrawn during growth, filtered and the cells resuspended in minimal medium. The reaction was started by adding PNPG (1×10^{-3} M) and terminated by the addition of 1 ml of 2 M Na_2CO_3 . The liberated aglycone was determined at 420 nm using a Spectronic 88 spectrophotometer. Controls without substrate were included and the absorbance obtained was subtracted. Carbon source during growth: 0.5% αMG (●); 0.5% maltose (□); 0.5% turanose (×); 0.5% trehalose (○); 0.5% sucrose (△).

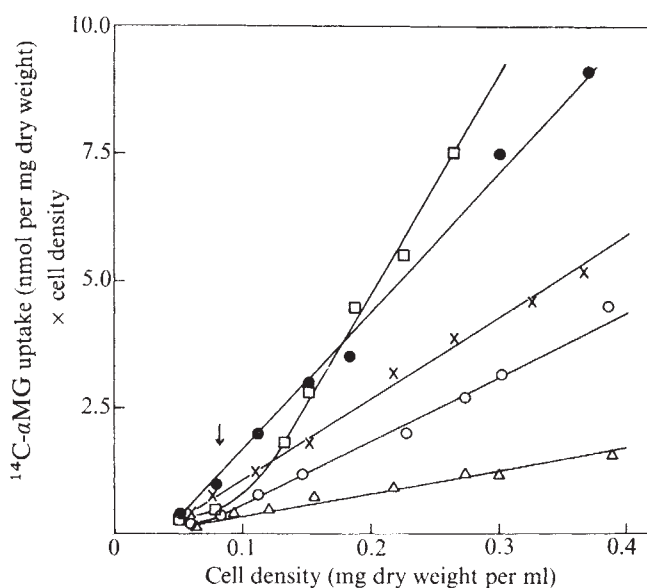


Fig. 4 Induction of ^{14}C - αMG uptake by the thermophilic bacillus. Cultures were grown in TL-minimal medium at 58°C . Samples were withdrawn during growth, filtered and suspended in the minimal medium lacking the carbon source. Duration of uptake 0.5 min. The ^{14}C - αMG concentration was 5×10^{-5} M, specific activity $3 \mu\text{Ci } \mu\text{mol}^{-1}$. Carbon sources during growth, 0.5% Na-succinate (△), 0.5% Na-succinate + 0.5% αMG (○), 0.5% αMG (×), 0.5% glucose (●), 0.5% Na-succinate + 0.5% glucose (□). Arrows show the time of addition of inducer (αMG or glucose) to succinate-grown cultures. For further details see legend to Table 1 and ref. 3.

α MG transport system was, however, much higher in the presence of glucose than in the presence of α MG.

It has already been pointed out by Harris and Kornberg¹ that glucose and α MG differ in their uptake properties. Our results accentuate these differences and show clearly that both the α MG phosphotransferase and the α MG transport system can be induced by α MG and not only by glucose. Although both glucose and α MG induce the uptake of α MG, the α MG-hydrolysing activity is induced by α MG and maltose, but not by glucose. The possibility, therefore, exists that the transport system induced by α MG is different from the one induced by glucose. A study of the specificity of the two inducible transport systems may clarify this point. The existence of an α -glucosidase in various yeast cells is well established^{5,6}. In *Saccharomyces cerevisiae* the simultaneous induction of two specific α -glucosidases, that is maltase and α MG-splitting enzyme, by either maltose or α MG have been reported⁷.

It remains to be seen whether the α -glucosidases induced by maltose and α MG in the thermophilic bacillus possess different and specific enzymatic activities or they represent a single enzyme with a substrate specificity towards both maltose and α MG.

In summary, our results clearly indicate that the thermophilic bacillus is able to catabolise α MG after it is transported into the cells, although α MG-splitting activity seems rather rare in prokaryotic organisms⁸. This report also demonstrates the ability of α MG to induce its own transport across the cell membrane. The substrates of the α -glucosidase activity, the optimal conditions for the enzymatic hydrolysis, and the reaction products formed, are being studied.

Note added in proof: Similar findings were recently reported with the mesophilic *Bacillus subtilis*⁹.

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¹ Harris, P., and Kornberg, H. L., *Proc. R. Soc.*, **B182**, 159–170 (1972).

² Epstein, I., and Grossowicz, N., *J. Bact.*, **99**, 414–417 (1969).

³ Reizer, J., and Grossowicz, N., *J. Bact.*, **118**, 414–424 (1974).

⁴ Freeze, E., Klotat, W., and Galliers, E., *Biochim. biophys. Acta*, **222**, 265–289 (1970).

⁵ Halvorsen, H., *Meth. Enzymol.*, **8**, 559–562 (1966).

⁶ Gorman, J., and Halvorsen, H., *Meth. Enzymol.*, **8**, 562–565 (1966).

⁷ Khan, N. A., and Eaton, N. R., *Biochim. biophys. Acta*, **146**, 173–180 (1967).

⁸ Bhuriratana, A., and Costilow, R. N., *Can. J. Microbiol.*, **19**, 169–176 (1973).

⁹ Schmalreck, A. F., Losher, H., and Teuber, M., *Biochem. Soc. Trans.*, **3**, 1004–1006 (1975).

malformin activity is inhibited by reducing agents¹² and it reacts with sulphhydryl compounds¹³, involvement of wall sulphhydryl groups in the activity of malformin was also proposed⁷. We report here on the effect of hydroxyproline and proline, amino acids important in the synthesis of extensin, on the growth-stimulating and root-curving activities of malformin.

Cuttings from 4-d-old green seedlings of *P. aureus* Roxb., grown in vermiculite, were obtained by excision 7.0 cm below the apical bud. The cuttings were transferred randomly to 12-ml vials containing 6.0 ml of test solution (12 cuttings per vial), and incubated in continuous light (1.35×10^4 erg cm⁻² s⁻¹, Champion F90T17/w, white fluorescent, 27–29° C). Deionised water was added daily to maintain the volume at approximately 6.0 ml. After 2 d the stem length was measured. Measurements from each vial were averaged and considered as a single determination. The growth increment ratio (GIR) was determined by dividing the growth increment of treated cuttings by that of controls (water treated). Malformin-induced root curvatures were determined by germinating seeds of *Zea mays* L. WF9 $\times$ 38-11 for 3 d in Petri dishes containing a sheet of Whatman No. 1 filter paper (9.0 cm) moistened with 3.0 ml of test solution, and counting the number of roots with at least a 90° C curvature from the original direction of growth².

Table 1 Effect of L-proline and L-hydroxyproline on growth stimulation and root curvature induced by malformin

Treatment	<i>Phaseolus aureus</i> growth stimulation (GIR)*	<i>Zea mays</i> root curvature (%)†
H ₂ O	—	1.4 $\pm$ 1.3
Malformin 0.1 μ M	1.64 $\pm$ 0.06	57.8 $\pm$ 11.8
Malformin 0.1 μ M, proline 10 mM	1.42 $\pm$ 0.04	60.7 $\pm$ 10.9
Malformin 0.1 μ M, hydroxyproline 10 mM	1.11 $\pm$ 0.05	14.3 $\pm$ 6.4
Proline 10 mM	0.86 $\pm$ 0.06	2.8 $\pm$ 2.8
Hydroxyproline 10 mM	0.82 $\pm$ 0.05	0.0 $\pm$ —

*Growth increment ratio = growth increment of treated cuttings/growth increment of controls (H₂O); $\pm$ s.e.

†Percentage roots with at least a 90° curvature; $\pm$ s.e.

L-Hydroxyproline markedly inhibited plant growth stimulation and root curvatures induced by malformin, but L-proline had little or no effect (Table 1). Although proline and hydroxyproline both inhibited growth of cuttings when applied alone, only hydroxyproline substantially reduced malformin-induced growth stimulation. Reduction of malformin-induced growth by proline was only slightly greater than that which occurred in the absence of malformin, but that of hydroxyproline was considerably greater. Neither of these amino acids alone stimulated growth or induced root curvatures. L-Leucine, DL-threonine, L-tyrosine, and DL-serine had little effect on malformin-induced growth stimulation, and in earlier experiments L-valine, L-isoleucine, and L-leucine did not inhibit curvatures induced by malformin¹². We concluded that the inhibition was specific for L-hydroxyproline. Although lower concentrations (1.0, 0.1 mM) of hydroxyproline were effective in inhibiting malformin-induced root curvatures, they had little effect on malformin-induced growth stimulation. Growth changes induced by malformin in corn roots were attributed primarily to effects on cell elongation⁶. In preliminary experiments L-hydroxyproline also reduced the severity of malformations on seedlings of *P. vulgaris* treated with malformin. Malformations on *P. vulgaris* have been described as the result of a change in the orientation of developing cells so that they elongate at an angle to the longitudinal axis of the plant¹⁴.

Inhibition of malformin activity by hydroxyproline and restoration by proline

MALFORMIN, (cyclo-D-cysteinyl-D-cysteinyl-L-valyl-D-leucyl-L-isoleucyl)¹, is produced by the fungus *Aspergillus niger* van Tiegh., induces severe malformations on stems and petioles of various plants, and causes root curvature². When ¹⁴C-malformin was supplied to cuttings of the bean *Phaseolus vulgaris* L. most of the ¹⁴C was found in the wall fraction and was liberated from a protein-like component in the walls as ¹⁴C-malformic acid (SO₃H-malformin) by oxidation³. Previous results indicated that malformin inhibited wall synthesis and altered wall composition^{4–6}. We recently found that malformin is a potent stimulator of plant growth and inhibits several phytochrome- and ethylene-mediated responses. Because some effects of light and ethylene on plant growth have been attributed to an increase in extensin^{8–11}, a hydroxyproline-rich protein in plant walls, it was suggested that malformin may alter the synthesis or function of this wall component⁷. Because

Table 2 Restoration of malformin activity by L-proline in the presence of an inhibitory concentration of L-hydroxyproline

Treatment	<i>Phaseolus aureus</i> growth stimulation (GIR)	<i>Zea mays</i> root curvature (%)
H ₂ O	—	0.5 ± 0.1
Malformin 0.1 µM	1.63 ± 0.04	51.6 ± 4.2
Malformin 0.1 µM, hydroxyproline 10 mM	0.97 ± 0.08	22.9 ± 6.5
Malformin 0.1 µM, hydroxyproline 10 mM, proline 10 mM	1.45 ± 0.05	48.2 ± 4.8
Hydroxyproline 10 mM, proline 10 mM	0.84 ± 0.07	3.1 ± 2.2

Table 3 Proline, hydroxyproline and total amino acid content of cell walls from normal and malformed internodes of *P. vulgaris**

Internode	Total amino acid	Hydroxyproline	Proline	Hydroxyproline Proline
Normal	21.2 ± 1.6	0.70 ± 0.05	0.68 ± 0.06	1.03 ± 0.17
Malformed	28.6 ± 1.7	2.68 ± 0.12	1.14 ± 0.02	2.35 ± 0.06

*Results are µg amino acid per mg dry weight cell wall ± s.e. Seedlings of *P. vulgaris* cv. Harvester were treated on the apical bud with malformin (10^{-5} M) as described². After 11 d the first internodes above the simple leaves were diced and frozen (-15°C) until analysed. Frozen material was homogenised for 5 min in 3:1 v/w 0.2 M Tris buffer (pH 7.5) and centrifuged at 150g for 10 min. The pellet was suspended in 10 ml Tris buffer, homogenised again and centrifuged as described. The cell wall pellet was washed twice with buffer (10 ml) and 3 times with deionised water (10 ml). After each wash the wall fraction was pelleted at 150g for 10 min. The wall pellet was washed with trichloroacetic acid (5%, 10 min, 90°C), cold trichloroacetic acid (5%, 5 min, 10 ml), 95% ethanol (70°C), ethanol-ether- CHCl_3 (2:2:1, 55°C) and ether-acetone (2:1, 25°C). After each wash the wall fraction was centrifuged (1,500g, 5 min)¹⁸. The cell walls were air dried, hydrolysed for 24 h by refluxing in 6N HCl (4,000:1 excess HCl) in a CO_2 atmosphere, and the hydrolysate cleared of humin¹⁹ by charcoal and filtration. The hydrolysate was dried *in vacuo* at 60°C , dissolved in deionised water, and analysed for hydroxyproline²⁰, proline²¹, and total amino acids²².

Proline rather than hydroxyproline is the usual precursor of bound hydroxyproline in plants^{11,15}, and the inhibition of cell elongation in the *Avena* coleoptile by hydroxyproline was suggested to result from an inhibition of the formation or utilisation of a hydroxyproline-rich protein in cell walls¹⁶. Growth inhibitory concentrations (0.5 mM) of hydroxyproline are incorporated directly into coleoptile proteins and proline prevents this incorporation¹⁷. The ability of proline to restore malformin activity in the presence of inhibitory concentrations of hydroxyproline was examined (Table 2). L-Proline almost completely restored the biological activity of malformin with regard to growth stimulation and root curvatures, although proline itself or in combination with hydroxyproline neither stimulated growth nor induced root curvatures (Tables 1, 2). The amount of amino acid present when proline and hydroxyproline were combined with malformin was double (20 mM) that present when only hydroxyproline was present. To ensure that simply increasing the amount of amino acid did not account for restoration of malformin activity we also assayed malformin activity in the presence of 20 mM hydroxyproline. Malformin activity was not restored.

To determine if malformin alters the metabolism of proline and hydroxyproline we examined the content of these amino acids in cell-wall fractions from normal and malformed internodes of *P. vulgaris*. This tissue was selected because malformin was shown to inhibit wall synthesis and alter wall composition⁵, as well as attach to a protein-like component in wall fractions of *P. vulgaris* internodes³. On a dry weight basis the amount of total amino acids in wall fractions from malformed internodes was greater than that in similar fractions from normal tissues (Table 3). Malformin induced a marked increase in the amount of hydroxyproline and proline content of wall fractions and increased the hydroxyproline-proline ratio more than two-fold. Because malformin seems to have more than one mode of action, stimulates elongation in some systems⁷, but inhibits elongation in other systems², we are unable to offer an explanation for our results. Since malformin inhibits several phytochrome- and ethylene-mediated responses, the effect of malformin on hydroxyproline-rich wall protein probably differs from that proposed for phytochrome and ethylene^{9,10}. But inhibition of malformin

activity by hydroxyproline, restoration of activity by proline, and alterations in the hydroxyproline-proline ratio in cell-wall fractions from malformed internodes strongly implicates a role of hydroxyproline-rich protein in the biological activity of malformin.

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- Bodanszky, M., and Stahl, G. L., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2791-2794 (1974); *Bioorg. Chem.*, **4**, 93-105 (1975).
- Curtis, R. W., *Pl. Physiol.*, **Lancaster**, **33**, 17-22 (1958); **36**, 37-43 (1961); *Science*, **128**, 661-662 (1958).
- Ciarlante, D., and Curtis, R. W., in *Proc. 8th Int. Conf. Plant Growth Substances*, 396-403 (Hirokawa, Tokyo, 1974).
- Curtis, R. W., and Kandler, O., *Pl. Physiol.*, **Lancaster**, **37**, 691-695 (1962).
- Curtis, R. W., *Pl. Cell Physiol.*, **10**, 203-211 (1969).
- Izhar, S., Bevington, J., and Curtis, R. W., *Pl. Cell Physiol.*, **10**, 687-698 (1969).
- John, W. W., and Curtis, R. W., *Experientia*, **30**, 1392-1393 (1974), *Pl. Cell Physiol.*, **16**, 719-728 (1975).
- Cleland, R., and Karlsnes, A. M., *Pl. Physiol.*, **Lancaster**, **42**, 669-671 (1967).
- Ridge, I., and Osborne, D. J., *Nature new Biol.*, **229**, 205-208 (1971).
- Sadava, D., and Chrispeels, M. J., *Dev Biol.*, **30**, 49-55 (1973).
- Lampert, D., *Adv. bot. Res.*, **2**, 151-218 (1965).
- Suda, S., and Curtis, R. W., *Pl. Physiol.*, **Lancaster**, **39**, 904-906 (1964).
- Iriuchijima, S., and Curtis, R. W., *Phytochemistry*, **9**, 1199-1202 (1970).
- Postlethwait, S., and Curtis, R. W., *Am. J. Bot.*, **46**, 31-35 (1959).
- Steward, F. C., and Pollard, J. K., *Nature*, **182**, 828-832 (1958).
- Cleland, R., *Pl. Physiol.*, **Lancaster**, **42**, 271-274 (1967); **42**, 1165-1170 (1967).
- Cleland, R., and Olson, A. C., *Biochemistry*, **7**, 1745-1751 (1968).
- Rendina, G., *Experimental Methods in Modern Biochemistry*, **324** (Saunders, Philadelphia, 1971).
- Chao, H., and Dashke, W. V., *Ann. Bot.*, **37**, 95-105 (1973).
- Kivirikko, K. I., and Liesmaa, M., *Scand. J. clin. Lab. Invest.*, **11**, 128-133 (1959).
- Troll, W., and Lindsley, J., *J. biol. Chem.*, **215**, 655-660 (1955).
- Moore, S., and Stein, W., *J. biol. Chem.*, **176**, 367-388 (1948).

Parathyroid hormone-responsive adenylate cyclase in induced transplantable osteogenic rat sarcoma

AN apparent paradox prevents accurate assessment of the role of cyclic AMP in the development and maintenance of tumours. Although cell culture experiments indicate that malignant transformation is associated with decreased adenylate cyclase activity and intracellular cyclic AMP levels^{1,2}, the same is not true of *in vivo* studies with

tumours, in which a range of results have been obtained, from low³ to normal⁴ and elevated⁵ tumour cell cyclic AMP levels. We report here the development of a transplantable osteogenic sarcoma which has retained through many generations a parathyroid hormone-responsive adenylate cyclase, providing a system which can be used to evaluate the significance of hormone-responsive adenylate cyclase for tumour cell growth and differentiation.

Tumours were induced in Sprague-Dawley rats by injecting carrier-free ³²P-orthophosphate according to a schedule which has been shown⁶ to lead to a high incidence of osteogenic sarcoma development. After an initial intraperitoneal injection of 1 μ Ci per g body weight, six subsequent injections of 0.6 μ Ci per g body weight were given at 2-week intervals. After a lag period of 6–9 months tumours became palpable, usually in the tibiae or femora, in approximately 50% of treated animals. Some microscopic features of primary and transplanted tumours have been described by Geddes-Dwyer *et al.*⁷

All the rats used for tumour induction and subsequent transplantation were derived from one original litter. Tumour transplantation was carried out initially by forcing small pieces of tumour through 60-mesh stainless steel gauze, and injection into the flank of recipients. In later experiments with established tumours, cells were dispersed by collagenase and hyaluronidase digestion, counted in a Coulter counter, and injected (10^6 – 3×10^6 cells) subcutaneously into the flank. In these conditions tumour transplantation had virtually a 100% success rate. Tumours appeared 2–3 weeks after transplantation and rapidly grew to a large size requiring further transplantation or leading to the death of the animal within 6 weeks.

Although in the early stage of growth the cut surfaces of tumours have a fleshy appearance, calcification eventually occurs in all tumours, frequently with large calcified deposits but sometimes detectable only microscopically. Figure 1 illustrates the microscopical appearance of a transplanted tumour showing early calcification. The mineralisation is not related to necrosis, and takes place on an intercellular ground substance which can readily be shown, with appropriate staining, to consist of collagen and PAS-positive material, presumably mucopolysaccharide. The tumour used for the biochemical and histological experiments described here consisted of fairly uniform cells (Fig. 1) not showing the pleomorphism often seen in human osteogenic sarcoma. In spite of a search through many sections of lightly and heavily calcified tumour, no osteoclast-like cells were seen. Similarly, using electron microscopy the cells were of uniform morphology with prominent polyribosomes and intercellular collagen fibres.

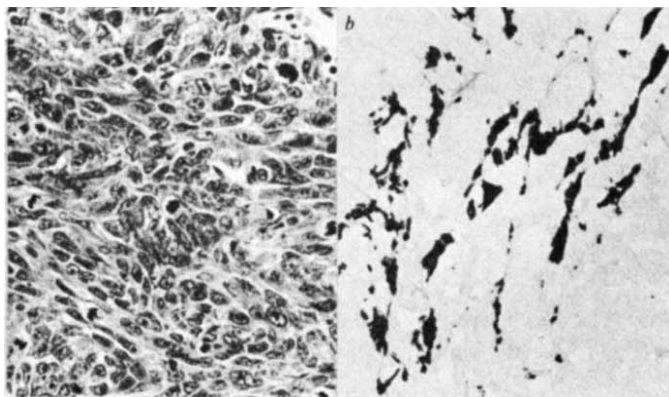


Fig. 1 Section of the tumour stained with haematoxylin and eosin (a) and by von Kossa's method (b). The tumour cells are slightly elongated, of relatively uniform morphology, and show much mitotic activity. Mineralisation of the intercellular matrix is shown by the dark areas in the von Kossa stained preparation ($\times 196$).

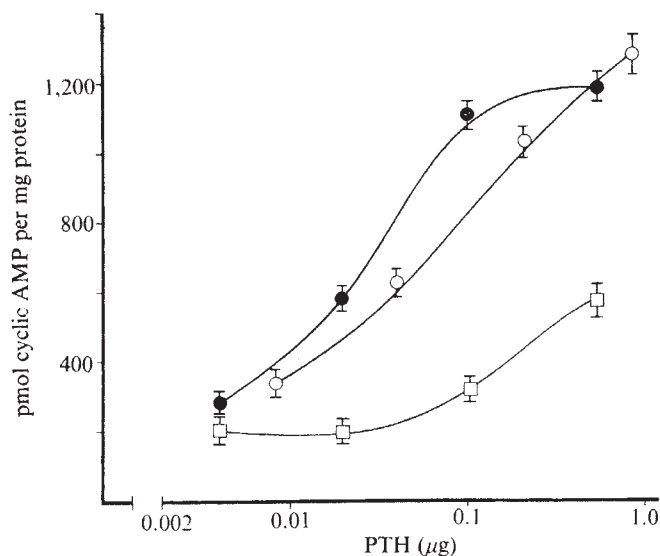


Fig. 2 Adenylate cyclase response of partially purified plasma membranes from rat osteogenic sarcoma on treatment with various preparations of PTH. Membranes were prepared and assay carried out as for Table 1. The hormone preparations used were as follows: ●, synthetic bovine PTH (1-34); □, synthetic bovine PTH (2-34); ○, highly purified bovine PTH, potency 2,500 U mg^{-1} (preparation 72/286, MRC Division of Biological Standards).

In all but those tumours in advanced stages of calcification dispersed cells could readily be prepared, and subcellular preparations made. The remaining experiments to be described were carried out with partially purified plasma membrane preparations, in which the adenylate cyclase activity of the tumour was studied. The response to various hormones is shown in Table 1. The major effect was obtained with parathyroid hormone (PTH) and lesser effects with prostaglandins E_1 and E_2 . Isoprenaline, glucagon, and salmon calcitonin stimulated adenylate cyclase to a small extent. The experiment of Fig. 2 illustrates that the interaction between PTH and the receptor in the sarcoma cell membranes resembles that between the hormone and its receptor in rat renal cortex membranes, in that parallel dose responses were obtained with highly purified bovine PTH and the synthetic peptide based on the first 34 amino acids of the hormone's sequence. Furthermore the synthetic (2-34) peptide had approximately the same relative effect in this system as it has been shown to have in rat renal membranes⁸. Oxidised PTH, which is ineffective biologically in other systems, had no stimulatory effect on adenylate cyclase in the tumour cell membranes. Thus the tumour seems to possess a PTH receptor which resembles in certain major features that present in the rat kidney. Although there is some information available regarding the response of bone cell adenylate cyclase to PTH⁹, it is not sufficiently complete to enable comparison with the present data.

These experiments were carried out on a tumour which had been transplanted almost 20 times over a period of 2 yr. Several other membrane preparations were examined, derived from other primary osteogenic tumours induced in the same way, and all were found to have a PTH-responsive adenylate cyclase. It is also important to note that in the tumour of this study no detectable change in the nature of the hormonal response took place during intensive study over a period of 6 months, encompassing six transplantations, and furthermore the hormone responsiveness remained constant through all stages of growth of individual tumours.

Changes in adenylate cyclase activity and its hormonal control have been observed in several experimental and

Table 1 Effect of various hormones on adenylate cyclase activity in partially purified plasma membranes from rat osteogenic sarcoma

Treatment	Concentration	Adenylate cyclase activity (pmol cyclic AMP formed per mg protein)*
Nil	—	55.8 ± 2.8
PTH	2 × 10 ⁻⁷ M	175.4 ± 4.9
	2 × 10 ⁻⁶ M	471.4 ± 1.9
Salmon calcitonin	3 × 10 ⁻⁷ M	69.5 ± 1.2
Glucagon	5 × 10 ⁻⁶ M	74.2 ± 1.8
Isoprenaline	10 ⁻⁵ M	98.4 ± 2.8
Prostaglandin E ₁	10 ⁻⁵ M	146.8 ± 4.1
Prostaglandin E ₂	10 ⁻⁵ M	138.4 ± 2.1
Prostaglandin F _{2α}	10 ⁻⁵ M	71.2 ± 1.7
Fluoride	10 ⁻² M	542.6 ± 35.8

Tumour was chopped and homogenised at 4 °C with six strokes of a loose fitting Dounce homogeniser (10 ml per g wet weight 0.25 M sucrose, 1 mM EDTA in 10 mM Tris-HCl, pH 7.4). Centrifugation was carried out in 50-ml tubes in the SS-34 head of a Sorvall RC2B centrifuge. The centrifuge was switched on, allowed to reach 2,000 r.p.m., and immediately slowed down with the brake applied. The supernatant was centrifuged for 10 min at 4,300 r.p.m. The upper, creamy layer from this pellet was removed in the same buffer and centrifuged at 6,000 r.p.m. for 10 min. The pellet was resuspended in the adenylate cyclase assay buffer and immediately assayed (50 µl suspension per assay tube). Protein was measured by the method of Hartree¹⁵. Adenylate cyclase incubations were carried out at 37 °C for 12 min. Assay mixtures consisted of the following components in a volume of 100 µl: 25 mM Tris-HCl, pH 7.4; 1 mM α-³²P-ATP (specific activity about 10 c.p.m. pmol⁻¹, Radiochemical Centre); 1 mM cyclic AMP; 2.5 mM phosphoenol pyruvate; 1.5 IU pyruvate kinase; 0.013% (w/v) bovine serum albumin; 30 mM KCl; 4.5 mM MgCl₂; 160 µg membrane protein; and hormone concentrations as indicated. Reaction was stopped by addition of 100 µl 40 mM ATP and boiling for 2 min. The generated ³²P-cyclic AMP was purified by the method of Salomon *et al.*¹⁶ as described previously¹⁷. Bovine PTH of potency 1,000 U per mg was used, and other hormones were as follows: salmon calcitonin (synthetic, gift of Armour Pharmaceuticals), prostaglandins E₁ and E₂ (gift of Dr J. E. Pike, Upjohn), and glucagon (Eli Lilly). In addition to the hormones indicated, the following had no significant effect on adenylate cyclase activity at the concentrations indicated: insulin (3 × 10⁻⁶ M); human growth hormone (3 × 10⁻⁶ M); ovine prolactin (3 × 10⁻⁶ M); pentagastrin (ICI, 10⁻⁵ M); arginine vasopressin (Sandoz, 10⁻⁵ M); human adrenocorticotrophic hormone (6 × 10⁻⁶ M).

*Results are means ± s.e.m. of triplicate determinations.

human tumours¹⁰⁻¹³ and in the premalignant stage of chemical carcinogenesis^{4,14}. The significance of these changes is not yet clear, and no unified concept could be developed at present. The hormone-responsive transplantable osteogenic sarcoma described here will facilitate testing of the effect of hormone-mediated changes in cyclic AMP on malignant cell proliferation. Moreover, differentiation of the tumour will be particularly amenable to study in view of its osteogenic capability, allowing it to progress from a soft tumour to one which has a network of mineralised matrix; this lends itself to relatively simple quantification.

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- Sheppard, J. R., *Nature new Biol.*, **236**, 14-16 (1972).
- Rein, A., Carchman, R. A., Johnson, G. S., and Pastan, I., *Biochem. biophys. Res. Commun.*, **52**, 899-904 (1973).
- Allen, D. O., Munshower, J., Morris, H. P., and Weber, C., *Cancer Res.*, **31**, 557-560 (1971).
- Boyd, H., Louis, C. J., and Martin, T. J., *Cancer Res.*, **34**, 1720-1725 (1974).
- Chayoth, R., Epstein, S. M., and Field, J. B., *Cancer Res.*, **33**, 1970-1974 (1973).
- Bensted, J. P. M., Blackett, N. M., and Lamerton, L. F., *Br. J. Radiol.*, **34**, 160-175 (1961).
- Geddes-Dwyer, V., Bosanquet, J. S., O'Grady, R. L., and Cameron, D. A., *Pathology*, **6**, 71-78 (1974).
- Tregear, G. W., *et al.*, *Endocrinology*, **93**, 1349-1353 (1973).
- Smith, D. M., and Johnston, C. C., Jr., *Endocrinology*, **95**, 130-139 (1974).
- Schorr, I., and Ney, R. L., *J. clin. Invest.*, **50**, 1295-1300 (1971).
- Schorr, I., Rathnam, P., Saxena, B. B., and Ney, R. L., *J. biol. Chem.*, **246**, 5806-5811 (1971).
- Goldfine, I. D., Roth, J., and Birnbaumer, L., *J. biol. Chem.*, **247**, 1,211-1,218 (1972).
- Martin, T. J., *et al.*, *Clinical Endocrinology*, **5**, suppl. 373S-386S.
- Christoffersen, T., Morland, J., Osnes, J. B., and Elgjo, K., *Biochim. biophys. Acta*, **279**, 363-366 (1972).
- Hartree, E. F., *Analyt. Biochem.*, **48**, 422-427 (1972).
- Salomon, Y., Londos, C., and Rodbell, M., *Analyt. Biochem.*, **58**, 541-548 (1974).
- Hunt, N. H., Martin, T. J., Michelangeli, V. P., and Eisman, J. A., *J. Endocr.* (in the press).

Reduced muscle acetylcholine sensitivity in rats immunised with acetylcholine receptor

ANIMALS immunised with acetylcholine receptor (AChR) purified from the electric organ of *Electrophorus electricus* or *Torpedo marmorata* develop a muscular weakness which is alleviated by injections of anticholinesterases¹⁻⁶. This observation has provided a valuable animal model for study of the disease myasthenia gravis. Sera from rats immunised with electric eel receptor contain antibody to AChR isolated from rat skeletal muscle^{2,6}, suggesting that the muscular weakness is due to a reduced endplate sensitivity to ACh. We report here a reduction in the ACh sensitivity of denervated muscles from immunised rats. Furthermore, there is a factor in the serum of immunised rats which can reduce the ACh sensitivity of denervated rat muscles *in vitro*.

Female Lewis rats (10 weeks old) were injected on a single occasion at multiple intradermal sites with 100 pmol of AChR protein, purified from the electric organ of *E. electricus*, in 0.1 ml of phosphate-buffered saline, emulsified in 0.1 ml of Freund's complete adjuvant. As additional adjuvant 2 × 10¹⁰ *Bacillus pertussis* organisms (special vaccine product, Eli Lilly) were injected subcutaneously. Rats immunised in this way develop two episodes of weakness; an initial transient episode after 8-9 d and a second chronic episode after 4 or more weeks.

Table 1 Reduction of extrajunctional ACh sensitivity of denervated hemidiaphragm muscle fibres by rat anti-AChR antiserum

Anti-rat ACh receptor titre (10 ⁻⁶ M)	Complement inactivation	Incubation period (min)	ACh sensitivity (serum treated) / ACh sensitivity (control) (%)
1.03	No	120	23*
1.53	No	45	28*
1.31	No	75	29*
1.61	Yes	120	29†
0.62	Yes	60	43‡
0.24	Yes	75	50*

In some experiments complement was inactivated by heating the serum for 30 min at 56 °C. Extrajunctional ACh sensitivities were measured in 15-20 fibres of 1-cm wide muscle strips before and after incubation with sera for 45-120 min at 37 °C. All sensitivity measurements were made at 21-23 °C. Anti-rat AChR titre is expressed as mol ¹²⁵I-α-cobratoxin binding sites precipitated per l of serum⁶. This assay in which rat antibody bound to ¹²⁵I-α-toxin-AChR is precipitated by rabbit anti-rat IgG, measures antibody directed against sites on the AChR molecule other than the ACh binding site. Statistical significance was determined using a *t* test.

* *P* < 0.0005.

† *P* < 0.025.

‡ *P* < 0.05.

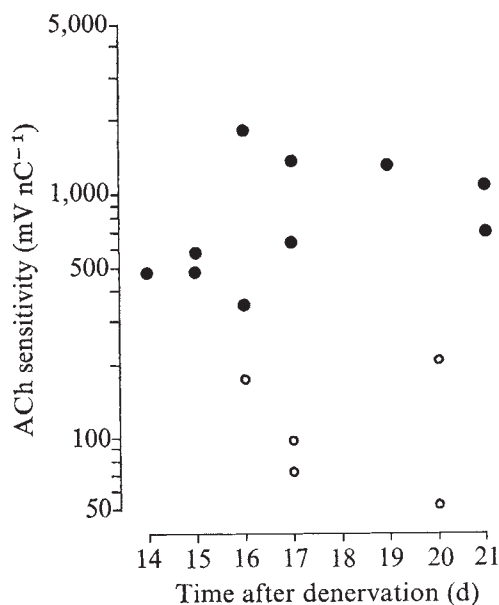


Fig. 1. Mean extrajunctional ACh sensitivities of denervated hemidiaphragm muscle fibres from adjuvant-treated rats (●) and rats immunised with AChR (○). Each point represents the mean sensitivity of 15–22 fibres measured at points 3–5 mm from the myotendinous junction. Extrajunctional ACh sensitivities were determined at room temperature (21–23 °C) by iontophoretic application of ACh from pipettes filled with 3 M AChCl (resistances 200–350 MΩ) positioned within 50 μm of the intracellular recording electrode. Sensitivities are expressed as mV nC⁻¹ (ref. 10), that is the amplitude of the ACh potential in mV per electric charge in nC passed through the iontophoretic pipette.

All experiments were made on animals during the second episode shortly after the appearance of generalised weakness. Control animals were injected with adjuvants only. Rat sera were tested for antibodies to rat AChR by immunoprecipitation using ¹²⁵I-α-cobratoxin-labelled receptor as described elsewhere⁶. Isolated hemidiaphragm muscles were examined electrophysiologically at room temperature (21–23 °C) in a modified Krebs solution (150 mM NaCl; 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 11 mM glucose, 5 mM HEPES-NaOH, pH 7.4, bubbled with 100% oxygen) using standard techniques.

As reported previously⁵, miniature endplate potential (m.e.p.p.) amplitudes were reduced in animals immunised with receptor. The mean m.e.p.p. amplitude at 20 control endplates was 0.74 ± 0.06 mV (mean $\pm$ s.e.m.) while m.e.p.p. amplitudes in muscles from immunised rats were usually less than 0.2 mV. The mean m.e.p.p. amplitude estimated at 34 endplates was 0.14 ± 0.01 mV. This value, however, probably overestimates the true amplitude, as m.e.p.p. amplitude histograms were often highly skewed, suggesting that smaller m.e.p.p.s. were lost in the recording noise level. Several properties of muscle fibres which affect m.e.p.p. amplitudes were similar in control and immunised rats. Muscle fibre resting potential and the reversal potential of the endplate potential showed little or no change. Also input resistance and membrane time constant in fibres of immunised rats (0.39 ± 0.02 MΩ (mean $\pm$ s.e.m., $n = 66$), 2.36 ± 0.08 ms ($n = 25$)) were not significantly different (t test, $P > 0.1$) from the values in control animals (0.37 ± 0.03 MΩ ($n = 57$), 2.22 ± 0.06 ms ($n = 34$)). The decrease in m.e.p.p. amplitudes is therefore likely to result either from a reduction in the size of the ACh quanta released from the nerve terminals or from a reduced postjunctional response to ACh. The latter possibility was examined by measuring the ACh sensitivity of extrajunctional regions of denervated muscles. Although extrajunctional receptors differ in some respects to junctional AChRs⁷, denervated muscle offers a convenient preparation for analysing changes in ACh sensitivity.

Hemidiaphragm muscle fibres were denervated under ether anaesthesia by sectioning the left phrenic nerve close to the muscle. Extrajunctional ACh sensitivities were determined after 2–3 weeks of denervation. The mean extrajunctional sensitivities of control denervated muscles ranged from 340–1,900 mV nC⁻¹, while muscles from immunised animals showed mean sensitivities of 51–200 mV nC⁻¹ (Fig. 1). Resting potential, input resistance and membrane time constants were similar in muscles from control and immunised rats denervated for an equivalent period. The lower ACh sensitivity in immunised rats must therefore result either from a reduction in the number of functional AChRs in the muscle membrane or from a reduction in the amplitude or time course of the conductance change associated with individual receptor activation.

The reduction in ACh sensitivity in muscles of immunised rats could be due to an alteration in the function of receptors by the binding of circulating antibodies. To test this idea, the effect of immune sera on the function of ACh receptors was studied *in vitro*. Extrajunctional ACh sensitivities of denervated hemidiaphragm muscle fibres were measured before and after incubation for 45–120 min with either control rat sera or sera from immunised rats. Immune rat sera reduced extrajunctional ACh sensitivities (Table 1), while sera from control rats inoculated with adjuvants were without effect. This finding suggests that the lower extrajunctional ACh sensitivity of denervated muscles in immunised rats is due to receptor modification by circulating anti-receptor antibodies. Since the antigenic determinants on AChRs that react with anti-receptor antibodies are common to both junctional and extrajunctional receptors⁸, the reduction of m.e.p.p. amplitudes in immunised animals may similarly be due to an antibody-mediated reduction in ACh sensitivity. This possibility is supported by the finding that sera from rabbits immunised with AChR can reduce m.e.p.p. amplitudes at frog motor endplates *in vitro*⁵.

Further information about the mode of action of anti-receptor antibodies must await the results of experiments using ACh noise analysis. These will show whether antibodies act by inactivating receptors in an 'all-or-none' manner, or by modifying the conductance change associated with individual receptor activations.

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- Patrick, J., and Lindstrom, J., *Science*, **180**, 871 (1973).
- Lennon, V. A., Lindstrom, J. M., and Seybold, M. E., *J. exp. Med.*, **141**, 1365 (1975).
- Sugiyama, H., Bendo, P., Meunier, J., and Changeux, J.-P., *FEBS Lett.*, **35**, 124 (1973).
- Heilbron, E., Mattsson, C., Stalberg, E., and Hilton-Brown, P., *J. neurol. Sci.*, **24**, 59 (1975).
- Green, D. P. L., Miledi, R., and Vincent, A., *Proc. R. Soc.*, **B189**, 57 (1975).
- Lindstrom, J., Lennon, V., Seybold, M., and Whittingham, S., *Ann. N.Y. Acad. Sci.* (in the press).
- Beranek, R., and Vyskocil, F., *J. Physiol., Lond.*, **188**, 53 (1967).
- Katz, B., and Miledi, R., *J. Physiol., Lond.*, **224**, 665 (1972).
- Brookes, J. P., and Hall, Z. W., *Biochemistry*, **14**, 2100 (1975).
- Miledi, R., *J. Physiol., Lond.*, **151**, 1 (1960).

Development of difference between red and white muscles in sensitivity to Ca^{2+} in the rabbit from embryo to adult

VARIOUS properties of the Ca^{2+} -activated troponin system are different in red (slow twitch) and white (fast twitch) muscle; these differences are in molecular weight^{1,2}, amino acid sequences of the subunits³, Ca^{2+} binding to troponin^{4,2}, and biochemical measures of contraction (actomyosin ATPase activity and superprecipitation^{4,7}). Although the preceding differences have been investigated, no report on physiological differences in Ca^{2+} -activated tension have been published. We compared the development of Ca^{2+} -activated tension in an oxidative muscle fibre (soleus) and that in a glycolytic muscle fibre (adductor magnus) of the rabbit from embryo to adult. We used a technique recently developed in our laboratory⁵ for mechanically disrupting the sarcolemma of skeletal muscle fibres. We then examined the data, in view of the known biochemical differences, for possible clues to the roles that troponins have in the Ca^{2+} sensitivity of the various types of muscle.

The sarcolemmas of fibres (~1 mm long) from the soleus, (red), and adductor magnus, (white), muscles of rabbits were disrupted by homogenisation as originally described by Kerrick and Krasner⁵. One red fibre and one white fibre in identical solutions were studied simultaneously. The fibres were mounted into stainless-steel clamps in a tension transducer similar to that used by Hellam and Podolsky⁶. They were then immersed in relaxing and contracting solutions, and their steady-state tensions were recorded⁷.

All solutions contained 70 mM of K^+ , 10^{-7} M of H^+ , 10^{-3} M of Mg^{2+} , 2×10^{-3} M of MgATP^{2-} , a total of 7×10^{-3} M of EGTA, and an ionic strength equal to 0.15. The major anions in the solutions were propionate and the $p\text{Ca}$ of the test solutions was varied from 8 to 3.6. The relaxing solutions had a calculated $p\text{Ca}$ of 8.0, and the maximally contracting solution

had a $p\text{Ca}$ of 3.6. Imidazole propionate was varied in all solutions such that ionic strength was held at 0.15. The total concentrations of K propionate, Mg propionate, Ca propionate, Na_2ATP , Na_2CP , and imidazole needed for the desired concentrations of the various ionic species and the ionic strength were determined by computer program⁸. The binding constants used in the program were taken from the literature⁸.

Data points for each of the $p\text{Cas}$ were determined and the means and standard errors of the means computed. In general, one data point for each $p\text{Ca}$ was collected for each fibre and several fibres were used. When a fibre broke or generated too small a force, a new fibre was mounted and studied.

The sensitivity of red muscle to Ca^{2+} was approximately 2.5 times greater than that of white muscle in the adult (Fig. 1a). In contrast, Ebashi *et al.* found that the calcium binding to troponin in white muscle was greater than in red. Both red and white muscle showed the same sensitivity to calcium ion in the embryo (Fig. 1c), but red muscle had about ten times greater sensitivity to $[\text{Sr}^{2+}]$ than white muscle in the adult (Fig. 1d). Sensitivity to calcium ion increases in red muscle with development from 26-d embryo to adult (Fig. 1b), but remains constant in white muscle (Fig. 1c). The difference in sensitivity between red muscle and white muscle develops from embryo to adult, primarily from increasing sensitivity to Ca^{2+} or Sr^{2+} (Fig. 1d) in the red muscle.

These data show that sensitivity to calcium ion and strontium ion changes during development in the soleus muscle but not in the adductor magnus muscle. Thus, if troponin is entirely responsible for muscle sensitivity to calcium ion, then changes in the troponin molecule itself must take place during development.

In contrast, studies of the biochemical measures of contraction have shown greater Ca^{2+} sensitivity of Ca^{2+} -activated ATPase activity and Ca^{2+} binding to troponin in white muscle than in red⁷. Thus, these differences are not in agreement with the differences in the biochemical measures of contraction⁷. The discrepancy between the two may be due to either disruption and isolation or removal of proteins, such as light chains of myosin.

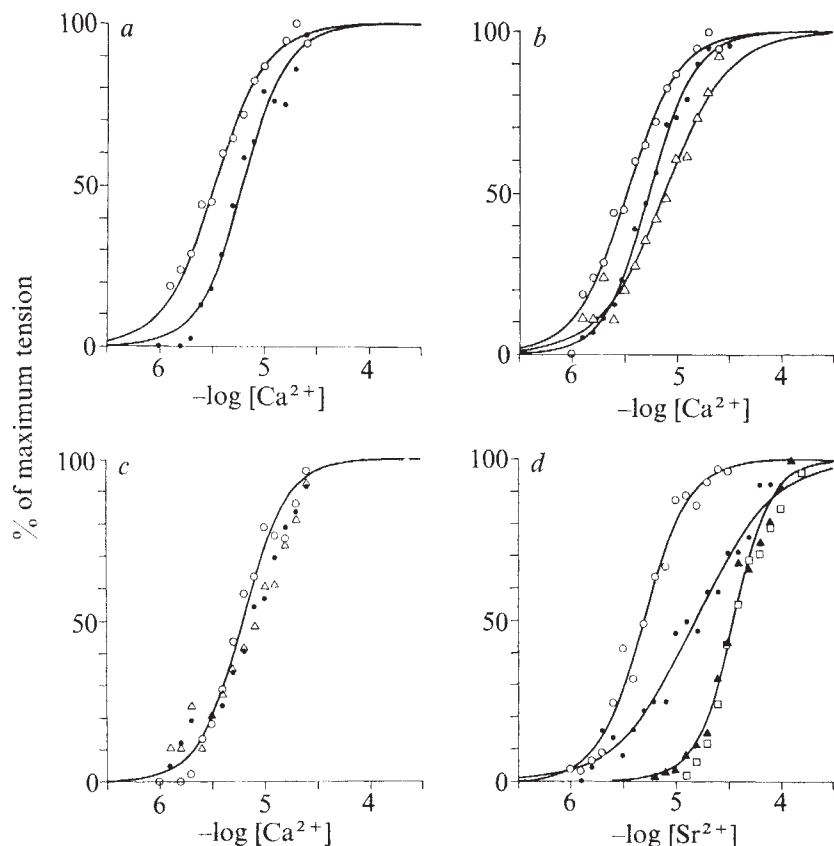


Fig. 1 The relationship between $p\text{Ca}$ or $p\text{Sr}$ ($-\log_{10}(\text{X}^{2+})$ (M) and percentage of maximum tension for mechanically disrupted rabbit adductor and soleus muscle fibres during development. Smooth curves were obtained by fitting the data to the Hill equation

$$\log_{10}\left(\frac{\%T}{100-\%T}\right) = n \log_{10}(\text{X}^{2+}) + R$$

where n and R are constants. *a*, $p\text{Ca}$ -tension relationship for adult rabbit soleus ($\circ$) ($n = 1.7$, $R = 9.6$) and adductor ($\bullet$) ($n = 2.0$ and $R = 10.5$). *b*, $p\text{Ca}$ -tension relationship for rabbit adult soleus ($\circ$) ($n = 1.7$, $R = 9.6$), 5-d-old soleus ($\bullet$) ($n = 2.0$, $R = 10.5$), and embryo soleus ($\triangle$) ($n = 1.4$, $R = 7.3$). *c*, $p\text{Ca}$ -tension for rabbit adult adductor ($\circ$) ($n = 2.0$, $R = 10.5$), 26-d embryo adductor ($\bullet$), and 26-d embryo soleus ($\triangle$). *d*, $p\text{Sr}$ -tension for rabbit adult soleus ($\circ$) ($n = 2.0$, $R = 10.5$) and adductor ($\square$) and 5-d-old soleus ($\bullet$) ($n = 1.2$, $R = 5.7$) and adductor ($\blacktriangle$) ($n = 2.3$, $R = 10.2$). The largest s.e.m. computed for any data point was $\pm 5.5\%$.

Unfortunately, no data have been published on the biochemical changes that occur in the troponin of the rabbit's red muscle and that of white muscle during development. Biochemical differences in subunits of troponin between red and white muscle have, however, been reported. One of these subunits (TNI) from white muscle inhibits Mg^{2+} -activated actomyosin ATPase activity of desensitised actomyosin more effectively than TNI from either red muscle or cardiac muscle. Studies of subunits from skeletal and heart muscles have also indicated differences in effect on actomyosin ATPase⁹. Because in the above studies researchers used incomplete contractile protein systems or preparations of more than one type of muscle, their experimental results cannot be related directly to the results reported in this paper.

Fluorescent antibody techniques show that three types of troponin and heavy chains of myosin (red slow, white fast, and cardiac) exist in individual chick muscle cells and that during development to adult only the troponin and myosin that are characteristic of a muscle type are retained¹⁰. One possible interpretation of these findings is that the troponin characteristic of white muscle may dominate the Ca^{2+} control of embryonic muscle, but is superseded during development by the troponin characteristic of red or cardiac muscle.

There is some evidence of a Ca^{2+} -sensitive regulatory system in the myosin molecule. It has been clearly demonstrated in scallops¹¹, Haselgrove¹² and Huxley¹³, using frog skeletal muscle that had been stretched so that there was no overlap of the actin and myosin filaments, demonstrated that the X-ray diffraction pattern associated with the myosin filaments changed when the muscles were electrically activated. Chaplain and Gergs¹⁴, using X-ray diffraction, noted that the cross bridges moved away from the thick filament on the addition of Ca^{2+} in adult glycerinated rabbit muscles stretched so that there was no overlap of thick and thin filaments. Further evidence for a conformational change of myosin comes from Morimoto and Harrington¹⁵. Using rabbit back muscles, they measured sedimentation coefficient and found it to change in a range from 10^{-6} to 10^{-5} M Ca^{2+} concentration; they then noted that the viscosity of myosin changed in this range of concentrations.

The light chains of myosin may modulate Ca^{2+} control of muscle since Ca^{2+} binding has been associated with the DTNB light chains of myosin in the rabbit¹⁴. The light chains of white muscle in the chick embryo are reported to be more similar to the light chains of white muscle in the adult than the red; this finding is consistent with our finding of unchanged Ca^{2+} sensitivity of white (adductor) muscle during development¹⁶. The myosin from white muscles show characteristic light chain patterns that are different from those of red muscles of the rabbit¹⁶; possibly, the development of light chains in red (soleus) muscle may have some role in the difference in and the change in development of Ca^{2+} sensitivity observed in this study.

We conclude that Ca^{2+} binding to troponin in red and white muscles does not entirely determine the sensitivity of these muscles to Ca^{2+} , in spite of indications by previous biochemical experiments; that differences either in the troponin molecule or in light chains of myosin, or other unknown factors are responsible for the difference in sensitivity of calcium between the two types of muscles; and that molecular changes from embryo to adult change the Ca^{2+} sensitivity in red muscle. These data show the importance of using muscles of one type in experiments designed to show the exact molecular mechanism for activation of a contraction by Ca^{2+} .

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- ¹ Syska, H., et al., *FEBS Lett.*, **40**, 253-257 (1974).
- ² Dabrowska, R., et al., *FEBS Lett.*, **29**, 239-242 (1973).
- ³ Van Eerd, J., *Biochem. biophys. Res. Commun.*, **64**, 122-127 (1975).
- ⁴ Ebashi, S., et al., *J. Biochem.*, **64**, 465-477 (1968).
- ⁵ Kerrick, W. G. L., and Krasner, B., *J. appl. Physiol.*, **39**, 1052-1055 (1975).
- ⁶ Hellam, D. C., and Podolsky, R. J., *J. Physiol., Lond.*, **200**, 807-819 (1969).
- ⁷ Ebashi, S., et al., *Q. Rev. Biophys.*, **2**, 351-384 (1969).
- ⁸ Donaldson, S. K. B., and Kerrick, W. G. L., *J. gen. Physiol.*, **66**, 427-444 (1975).
- ⁹ Tsukui, R., and Ebashi, S., *J. Biochem.*, **73**, 1119-1121 (1973).
- ¹⁰ Masaki, T., and Yoshizaki, C., *J. Biochem.*, **76**, 123-131 (1974).
- ¹¹ Szent-Györgyi, A. G., et al., *J. molec. Biol.*, **74**, 179-203 (1973).
- ¹² Haselgrove, J. G., thesis, Univ. Cambridge (1970).
- ¹³ Huxley, H. E., *Cold Spring Harb. Symp. quant. Bio.*, **37**, 361-376 (1973).
- ¹⁴ Chaplain, R. A., and Gergs, V., *Biochem. biophys. Res. Commun.*, **61**, 517-524 (1974).
- ¹⁵ Morimoto, K., and Harrington, W. F., *J. molec. Biol.*, **88**, 693-709 (1974).
- ¹⁶ Sreter, F., et al., *J. Cell Biol.*, **55**, 586-594 (1972).

Is chromatid-type damage in ataxia telangiectasia after irradiation at G_0 a consequence of defective repair?

THE radiosensitivity of patients with ataxia telangiectasia (AT) has been indicated by *in vivo* studies¹⁻³ and *in vitro* studies on skin fibroblasts⁴. We present data here to show that this radiosensitivity is observed at the chromosomal level in blood lymphocytes X irradiated at different stages of the cell cycle and for the same range of doses as our survival experiments⁴. Our results are consistent with the hypothesis that there is a defect in some form of DNA repair in these patients. From the observation of the types of chromosome aberrations seen in AT lymphocytes after irradiation at G_0 /early G_1 , we propose a mechanism for the origin of these aberrations, based on the premise that the G_0 /early G_1 chromosome contains a single DNA double helix, in which one polynucleotide chain can be damaged independently of the other.

From previous work on radiation cytogenetics we expected to see only chromosome-type damage (rings, dicentric and fragments) in the mitosis following G_0 irradiation⁵. These observations have led to the concept that the chromosome at this phase responds to X irradiation as though it were a single linear entity⁶. Chromatid-type damage according to this theory is seen after irradiation in late G_1 , S and G_2 .

Heparinised blood was obtained from four AT patients and five controls. Table 1 shows the results of full chromosome analyses of unirradiated lymphocytes. Spontaneous chromosome aberrations are a recognised feature of AT⁷. Although gaps and breaks are fewer in these four patients than in others previously reported⁷, the number of cells with non-clonal rearrangements is greater in AT patients than in normals. (Two AT patients show the presence of a lymphocyte clone.) Irradiated lymphocytes were analysed for the presence of chromosome- and chromatid-type aberrations. The results of irradiation with 200 and 400 rad in G_0 are shown in Table 2. AT lymphocytes do not show rings and dicentric at a consistently higher frequency than controls, but the total number of fragments (terminal and interstitial deletions) is greater in AT cells. It is accepted that one fragment is associated with each major exchange⁸, but allowing for incomplete exchanges, the ratio of fragments to rings and dicentric is expected to be greater than unity. In our controls this ratio was 1.59 after each dose of radiation. The ratio was higher in AT cells and increased with larger X-ray doses (1.77 at 200 rad and 2.44 at 400 rad). In AT cells therefore there is a large excess of deletions which are not associated with chromosome-type exchanges.

Our results also show that AT cells exhibit chromatid-type damage when irradiated in G_0 . This is seen as a large number of gaps and breaks, but most striking is the appearance of numerous chromatid interchanges, particularly triradials (Fig. 1a). The presence of these interchanges at

Table 1 Chromosome analysis of lymphocytes from AT and controls

Patients	Sex	No. of cells analysed	Total no. of rings and dicentrics	Total no. of fragments	Total no. of gaps and breaks	No. of cells with stable changes	Total no. of abnormal cells	Clone present
AT								
AT 4BI	M	100	0	0	17	6	19	—
AT 5BI	M	100	24	2	5	18	38	+
AT 6BI	F	100	2	3	3	16	24	+
AT 7BI	F	100	1	2	11	5	17	—
Controls								
CON 1BI	M	100	1	0	14	2	15	—
CON 2BI	F	100	0	1	8	1	10	—
CON 3BI	M	100	0	2	5	1	8	—
CON 4BI	F	100	0	0	8	1	8	—

0.4 ml blood was cultured in 4.0 ml Ham's F10 medium supplemented with 10% bovine serum, 1% phytohaemagglutinin, penicillin and streptomycin (100 IU ml⁻¹). Unirradiated lymphocytes were fixed after 48 h in culture, during their first division.

a high frequency after G₀ irradiation clearly distinguishes AT cells from controls. Chromatid-type damage of any kind is rarely seen in normal lymphocytes irradiated at G₀.

Irradiation during G₂ induced a very high level of chromatid gaps and breaks in AT lymphocytes (Fig. 1b) compared with controls (Table 3), agreeing with a previous report⁹. The frequency of chromatid interchanges was again higher in AT cells after both radiation doses, although interchanges were also present in irradiated controls at G₂. So far we have no data on subchromatid aberrations which can be observed at the second mitosis after irradiation at G₂.

We investigated the possibility that some AT lymphocytes showing triradial formation after irradiation at the time of culture initiation, were in G₂ and not in G₀. By scanning lymphocytes from uncultured blood on a Vickers microdensitometer, we found that the DNA content in all cells (from two AT patients and two controls) was consistent with their being in G₀. Cells in G₂ were used as positive controls. This evidence, together with the fact that unstimulated lymphocytes cultured for 48 h showed no mitoses, suggests that all lymphocytes from the AT patients

were in G₀ at the time of irradiation. We believe, therefore, our observations show that chromatid damage by X rays is not restricted to late G₁, S or G₂ in AT cells, but can occur after irradiation at G₀.

To explain the types of chromosome aberration seen in both AT and normal cells after irradiation at G₀ we propose that the chromosome at G₀ and in early G₁ behaves as a double-stranded structure. While we appreciate the difficulties of extrapolating from observations on chromosomes to the molecular level, we suggest that the observations are consistent with the basic unit being the DNA double helix. X rays can resolve the two strands at G₀, producing single-strand damage (breaks or base damage). In normal cells such damage is usually repaired before S, since chromatid aberrations are rarely seen at mitosis. The chromatid-type damage after late G₁ irradiation may be a consequence of insufficient time between G₁ and S phase for single-strand damage to be repaired (as Bender suggested¹¹). In AT cells, however, we suggest that much of the damage to single strands of DNA is not repaired and persists to manifest itself as chromatid-type damage (including triradials) at the next mitosis. Double-strand damage produces chromosome-type aberrations after G₀

Table 2 Chromosome analysis after X irradiation in G₀

Patients	Ring	Dic.	Frag.	tg	tb	cg	cb	Tric.	Tid. frag.	Chromatid interchanges		
										Trirad.	Quadri.	Other
200 rad												
AT												
AT 4BI	11	29	103	11	5	4	0	0	0	4	0	0
AT 5BI	14	44	79	11	6	2	1	0	1	7	1	0
AT 6BI	6	29	62	6	3	5	2	0	2	0	1	0
AT 7BI	9	32	64	14	1	0	0	1	0	2	1	0
Controls												
CON 1BI	6	25	34	11	0	3	0	0	0	0	0	0
CON 2BI	ND											
CON 3BI	5	22	47	8	1	0	0	0	0	1	0	0
CON 4BI	0	29	61	6	0	1	0	0	0	0	0	0
400 rad												
AT												
AT 4BI	18	75	264	21	18	3	1	5	0	12	1	2
AT 5BI	19	79	217	38	20	7	2	3	6	8	0	2
AT 6BI*	10	37	75	6	4	0	0	1	5	0	0	0
AT 7BI	20	66	235	32	19	2	0	0	0	14	2	4
Control												
CON 1BI	18	77	145	8	1	0	0	8	0	1	0	0
CON 2BI	10	95	167	4	2	3	0	3	1	1	0	0
CON 3BI	10	90	170	2	1	2	0	1	0	0	0	0
CON 4BI	14	78	142	3	1	2	1	1	4	0	0	0

ND, Not done; Ring, rings; Dic., dicentrics; Frag, fragments; tg, chromatid gaps; tb, chromatid breaks; cg, chromosome gaps; cb, chromosome breaks; Tric., tracentrics; Tid. frag, chromatid fragments; Trirad., triradials; Quadri., quadriradials.

*Only 22 cells analysed.

X irradiation was carried out using a Pantak X-ray machine (300 keV, 12 mA, half value thickness 1.5 mm Cu, FSD 50 cm, 180 rad min⁻¹). Whole blood was irradiated at G₀, that is, immediately before being put into culture. Doses of 200 and 400 rad were used. The lymphocytes were collected in their first post-irradiation division at 52 h and 50 cells were analysed for each dose used.



Fig. 1 *a*, Triradial chromosome (chromatid-type damage) together with dicentrics (chromosome-type damage) in an AT lymphocyte irradiated with 400 rad at G_0 . *b*, Level of chromatid damage (gaps and breaks) in an AT lymphocyte irradiated with 200 rad at G_2 .

irradiation, and chromatid damage after G_2 in normals. The high level of deletions after G_0 irradiation and chromatid-type damage after G_2 in AT cells, implies either a high level of double-strand breakage or failure to rejoin. Using alkaline and neutral sucrose gradients, however, we have previously shown normal single-strand rejoining and also, we believe, normal double-strand rejoining in AT cells⁴. The enhanced levels of both types of chromosome aberrations in AT cells after normally sublethal doses of X

irradiation may be a consequence of a failure to excise lesions in the DNA. Paterson *et al.*¹⁰ have confirmed that AT cells show normal single-strand rejoining and, using supra-lethal doses of γ rays, have shown that AT cells are defective in excision repair of γ -ray induced base damage.

Many reports¹¹⁻¹⁶ have speculated on the possibility that incomplete repair mechanisms facilitate resolution of molecular damage at the chromosomal level, including aberrations induced by ultraviolet light and chemical compounds. The possible role of failure to repair damage in the production of chromosome aberrations has been shown in cells from patients with xeroderma pigmentosum (XP)¹⁷. The high incidence of malignancies in both AT and XP may be a consequence of the defective DNA repair in both of these syndromes, giving rise to cytogenetically abnormal cells with a potential to become malignant.

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- 1 Gotoff, S. P., Amirmokri, E., and Leibner, E. J., *Am. J. Dis. Childh.*, **114**, 617-625 (1967).
- 2 Morgan, J. L., Holcomb, T. M., and Morrissey, R. W., *Am. J. Dis. Childh.*, **116**, 557-558 (1968).
- 3 Cunliffe, P. N., Mann, J. R., Cameron, A. H., Roberts, K. D., and Ward, H. W. C., *Br. J. Radiol.*, **48**, 374-376 (1975).
- 4 Taylor, A. M. R., *et al.*, *Nature*, **258**, 427-429 (1975).
- 5 Evans, H. J., in *Chromosomes and Cancer* (edit. by German, J.), 191-237 (Wiley, New York, 1975).
- 6 Wolff, S., *Mutat. Res.*, **8**, 207-214 (1969).
- 7 Oxford, J. M., Harnden, D. G., Parrington, J. M., and Delhanty, J. D., *J. med. Genet.*, **12**, 251-262 (1975).
- 8 United Nations Scientific Committee on the Effects of Atomic Radiation, *Official records of the General Assembly*, **24**, Suppl. 13 (A/7613) (1969).
- 9 Rary, J. M., Bender, M. A., and Kelly, T. E., *Am. J. Human Genet.*, **26**, 70A (1974).
- 10 Paterson, M. C., Smith, B. P., Lohman, P. H. M., Anderson, A. K., and Fishman, L., *Nature*, **260**, 444-446 (1976).
- 11 Bender, M. A., Griggs, H. G., and Bedford, J. S., *Mutat. Res.*, **23**, 197-212 (1974).
- 12 Evans, H. J., and Scott, D., *Proc. R. Soc.*, **B**, **173**, 491-512 (1969).
- 13 Kihlman, B. A., *Actions of Chemicals on Dividing Cells* (Prentice Hall, Englewood Cliffs, New Jersey, 1966).
- 14 Stich, H. F., and San, R. H. C., *Mutat. Res.*, **10**, 389-404 (1970).
- 15 Evans, H. J., in *Genetical Aspects of Radiosensitivity: Mechanisms of Repair*, 31-48 (International Atomic Energy Agency, Vienna, 1966).
- 16 Bender, M. A., Griggs, H. G., and Walker, P. L., *Mutat. Res.*, **20**, 387-402 (1973).
- 17 Sasaki, M. S., *Mutat. Res.*, **20**, 291-293 (1973).

Table 3 Chromosome analysis after X irradiation in G_2

Patients	Ring	Dic.	Frag.	tg	tb	cg	cb	Tric.	Tid. frag.	Chromatid interchanges		
										Trirad.	Quadri.	Other
100 rad												
AT												
AT 5BI	0	10	12	93	44	2	0	0	4	5	2	0
AT 6BI	0	1	5	113	41	0	0	0	11	3	3	0
Controls												
CON 2BI	0	1	2	14	12	0	0	0	0	0	4	0
CON 5BI	0	0	1	14	3	1	0	0	0	0	1	0
200 rad												
AT												
AT 5BI	0	9	26	146	102	4	3	1	6	14	2	3
AT 6BI*	0	1	6	119	61	2	0	0	3	1	7	0
Controls												
CON 2BI	0	1	4	15	10	1	0	0	2	0	1	1
CON 5BI	0	0	2	18	9	3	0	0	0	3	1	0

*Only 25 cells analysed.

Lymphocytes in G_2 were irradiated for 4 h before collecting at 52 h. 50 cells were analysed per person for each dose.

Defective excision repair of γ -ray-damaged DNA in human (ataxia telangiectasia) fibroblasts

ATAXIA telangiectasia (AT) (Louis-Bar syndrome) is a rare human neurovascular disease displaying an autosomal recessive pattern of inheritance¹⁻³. Affected individuals, although clinically normal at birth, symptomatically develop cerebellar ataxia (loss of muscular coordination) and oculocutaneous telangiectasis (chronic dilation of the small blood vessels) in early childhood. The course of the disease follows a variable progression commonly leading to total neurological incapacitation before puberty. Accessory complications include lymphoreticular neoplasia^{2,3}, bronchiectasis^{1,2}, recurrent sinopulmonary infections^{1,2}, decreased levels of serum immunoglobulins IgA and IgE (refs 3 and 4), impaired cellular immunity^{3,4}, and widespread chromosomal instability⁵. AT patients, on receiving conventional radiotherapy for tumour treatment, tend to develop unusually severe complications often culminating in premature death⁶⁻⁸. Pronounced radiosensitivity is also observed at the cellular level in laboratory studies; the number of radiation-induced chromosomal aberrations is enhanced in leukocytes obtained from AT donors⁹. Moreover, diploid fibroblasts cultured from affected individuals exhibit a reduced ability to form colonies following exposure to γ radiation¹⁰ and radiomimetic chemicals¹¹. Since the principal damage induced by both types of agents occurs in the DNA and seems to be acted on by the same enzymatic repair mechanisms^{12,13}, it would seem probable that the molecular basis for the clinical radiosensitivity of AT patients stems from a deficient DNA repair mechanism. We therefore measured the DNA repair properties of AT fibroblasts after exposure to γ radiation. Data presented below provide direct biochemical evidence that diploid strains from AT donors are indeed impaired in DNA repair; in particular, these cell lines possess an enzymatic defect in an excision-type repair process operating on γ -modified nitrogenous base residues.

The ability of AT strains to carry out two repair processes, rejoining of γ -induced single-strand breaks (that is, scissions in the sugar-phosphate backbone of individual polynucleotide chains) and excision of γ -modified base residues, was evaluated with the aid of two complementary physicochemical techniques: (1) *in vitro* enzymatic assay^{14,15} and (2) equilibrium density-gradient centrifugation¹⁶. The enzymatic assay monitors the DNA separately for the restitution of strand breaks and for the disappearance of base defects, during *in vivo* incubation after γ irradiation. Base defects are detected as sites susceptible to γ endonuclease activity in a crude extract of the bacterium

*Micrococcus luteus*¹⁷ (referred to as γ endonuclease-susceptible sites). The equilibrium centrifugation technique measures the magnitude of radiation-induced repair replication; this parameter reflects the sum total of nucleotides inserted at damaged sites along each strand during the course of repairing both types of radioproducts¹⁸.

Our experiments were carried out with five (two control, three AT) human diploid strains: CRL 1141 and CRL 1147, cultured from two clinically normal volunteers; and AT3BI, CRL 1312, and CRL 1343, derived from three AT patients of unrelated kindred (for details, see Table 1).

To monitor the metabolic fate of γ radioproducts, irradiated cultures of normal (CRL 1141) and AT (AT3BI) strains were incubated and the numbers of single-strand breaks and γ endonuclease-susceptible sites remaining in the DNA at various times were measured (Fig. 1). The restitution of directly induced strand breaks in AT cells occurred with exponential kinetics indistinguishable from those displayed by normal cells. The other two AT strains, CRL 1312 and CRL 1343, also exhibited similar strand-rejoining kinetics (data not shown). Thus, in spite of their pronounced sensitivity to γ inactivation¹⁰, AT strains seem to possess a normal enzymatic mechanism for rejoining radiation-induced single-strand breaks. Others^{10,20} have independently arrived at the same conclusion.

In marked contrast to the efficient repair of strand breaks, the rate of disappearance of γ endonuclease-susceptible sites was more than four times slower in AT3BI than in CRL 1141 cells. For instance, whereas the normal strain achieved 80% site removal within 3 h, the mutant strain required twice as long to act on only 50% of the initial sites. In as much as (1) the strand-rejoining mechanism seems to proceed in a manner similar to the last three steps in excision repair (excision, repair synthesis, ligation), probably using similar, if not the same, enzymes^{12,13}; and (2) this rejoining process seems to be normal in AT strains, it seems reasonable that the time course of site disappearance reflects the first step in excision repair of γ -modified base residues *in vivo*. Thus, the simplest conclusion is that the AT3BI strain lacks a fully functional γ endonuclease considered to initiate this excision-repair process.

If the observed disappearance of γ endonuclease-susceptible sites indeed reflects an excision-repair process acting on γ -induced base damage, it follows that the impaired ability of AT3BI cells to effect site removal should be accompanied by a reduction in the magnitude of γ -stimulated repair replication. That is, a defective capacity to excise base defects should accordingly lead to a decrease in the number of gaps which need to be filled by repair synthesis. We therefore measured the amount of repair replication occurring in normal and AT strains during 2 h after irradiation (Fig. 2). The level of repair replication detected in two normal cell lines, CRL 1141 and

Table 1 General description and *in vitro* characteristics of diploid fibroblast strains from normal and AT donors*

Strain designation	Clinical description	Donor		<i>In vitro</i> age during assay†	Doubling time (h)
		Age (yr)	Sex		
CRL 1141 (Ba Del)	Normal	3	M	18-28	38-43
CRL 1147 (Ter Loy)	Normal	9	F	10-19	41-49
AT3BI	AT	4	M	26-34	39-44
CRL 1312 (Sa Gru)	AT	7	F	9-17	42-47
CRL 1343 (Se Pan)	AT	7	F	10-21	36-40

*Tissue origin: human skin.

†Expressed as cell population doublings since biopsy, although cultures were actually diluted 1:3 at each subcultivation.

AT3BI was provided by A. R. Lehmann, University of Sussex; the remaining four were obtained from the American Type Culture Collection, Rockville, Maryland. Using standard cell culture procedures, monolayer stocks of each fibroblast strain were routinely maintained at 37 °C in thymidine-free F12 medium¹⁹ supplemented with 15% (v/v) foetal calf serum (Gibco) and a penicillin-streptomycin mixture (100 IU each ml⁻¹). Unless otherwise indicated, all cell culture supplies were purchased from Microbiological Associates Inc.

Experimental cultures in late logarithmic growth phase were prepared by seeding $\approx 8 \times 10^5$ cells in plastic Petri dishes (15 cm diam., Lux Scientific) followed by incubation for 24-48 h at 37 °C. In this and subsequent experimental incubations the F12 medium contained newborn (10%, v/v) rather than foetal calf serum. Radiation treatment was administered at ice-bath temperature with either a, ⁶⁰Co γ rays (dose rate, 26-27 krad min⁻¹) delivered in a Gammacell 220 (Atomic Energy of Canada Ltd) or, in certain control studies, b, far ultraviolet radiation (chiefly 254-nm wavelength; incident fluence rate, 1.1 J m⁻² s⁻¹)¹⁴. Cultures were maintained under anoxia during γ irradiation by introducing a stream of N₂ gas into aspirated Petri dishes for 5 min before, and during the exposure. All remaining experimental manipulations and treatment schedules, including those involved in the *in vitro* enzymatic assay and equilibrium density-gradient centrifugation, were performed as described briefly in the figure captions and in detail elsewhere¹⁴⁻¹⁶.

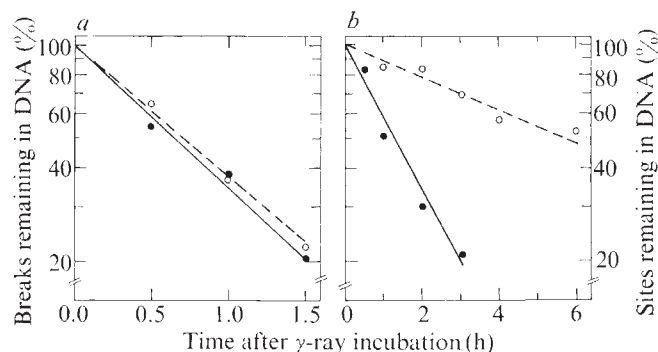


Fig. 1 Time-dependent disappearance of γ radioproducts from the DNA of normal (●, CRL 1141) and AT (○, AT3BI) fibroblasts exposed under anoxia to 50 krad ^{60}Co γ -radiation. *a*, Single-strand breaks and *b*, γ endonuclease-susceptible sites were monitored by an *in vitro* enzymatic assay similar to that described earlier for the quantitation of ultraviolet-induced pyrimidine dimers^{14,15}. The DNA of the cells was labelled for 24–48 h with either ^3H -methylthymidine (specific activity, 2 Ci mmol⁻¹) or ^{14}C -thymidine (specific activity, 45–53 mCi mmol⁻¹) (Amersham, Searle) added to the F12 incubation medium at a concentration of 1.0 $\mu\text{Ci ml}^{-1}$. After removing the radioactive medium, ^3H -labelled cultures of each strain were irradiated and immediately incubated for various times in parallel with ^{14}C -labelled, unirradiated (control) cultures. Parallel cultures were lysed and their ^3H - and ^{14}C -DNAs were coextracted *in toto*. Two samples of the purified DNA were then incubated for 30 min at 37 °C, one with, and the other without, a crude cell-free extract from *M. luteus* (the equivalent of fraction II of Carrier and Setlow²⁵). Typically, the 200- μl reaction solution contained: 2 μmol potassium phosphate (pH 7.5); 0.2 μmol EDTA; 0.2 μmol 2-mercaptoethanol; 1 μg ^3H -labelled, irradiated DNA ($\approx 15,000$ d.p.m.); 1 μg ^{14}C -labelled, unirradiated DNA (5,000 d.p.m.); 10 μg tRNA (General Biochemicals); 20 μg (protein) *M. luteus* extract (an amount in excess of that needed to complete the endonuclease reaction). At the completion of incubation 50 μl 2 N NaOH was added, and the samples were subjected to velocity sedimentation (40,000 r.p.m. at 20 °C for 135 min in a Spinco SW-56 rotor) in alkaline sucrose gradients (5–20%, w/v; 0.5 M NaCl; 0.21 N NaOH; 10 mM EDTA). After fractionation and liquid scintillation counting, computer analysis of the radioactivity distributions in the gradient profiles yielded the number of single-strand breaks in the irradiated, compared with non-irradiated, DNA. The value obtained for the DNA sample incubated alone is taken as the number of directly induced strand breaks remaining in the radiation-damaged DNA, whereas the additional breaks in the extract-treated sample represent the number of nuclease-susceptible sites. Data for each curve were normalised by expressing the lesion incidence in DNA from incubated cultures as a percentage of the corresponding value for the non-incubated culture, that is, the number initially produced per 10⁷ daltons DNA = 100% (≈ 0.8 single-strand breaks; ≈ 1.2 nuclease-susceptible sites). Experimental points represent the average of triplicate determinations (s.d. $\leq 13\%$).

CRL 1147, increased rapidly with γ -ray doses up to 75 krad at which stage the level seemed to approach a plateau value. Although the general response of three AT strains, including AT3BI, to radiation treatment was also dose dependent, the maximal extent of repair replication attained by each (45–60 d.p.m. per μg DNA) was only about half that (≈ 110 d.p.m. per μg DNA) found in the normal strains. It seems, therefore, that the level of repair replication attained in the AT strains after γ irradiation is lower than that achieved by the normal strains, providing additional evidence that AT strains are impaired in excision repair of γ -induced base defects.

It was crucial to establish further that the observed malfunction was specific and not an artefactual consequence of some systematic peculiarity in the general properties of the mutant strains; particularly as excision repair of γ radioproducts was not totally absent from the AT strains. Repair efficiency was independent of biological age, both *in vivo* (chronological age of donor at biopsy) and *in vitro* (age in culture). The repair deficiency in AT strains did not simply stem from a general depression in cell growth rate in culture. The population doubling times for the two normal and three mutant cell lines did not vary greatly, nor was a positive

correlation found between the kinetics of excision repair and growth rate (see Table 1). All strains were mycoplasma-free as judged by assaying exogenous ^3H -uracil/ ^3H -uridine incorporation into RNA²¹.

The response of AT strains to ultraviolet radiation was also monitored, since excision repair of ultraviolet photoproducts and γ radioproducts is thought to be mediated by parallel mechanisms¹³ which, except for specific endonucleases acting on the different ultraviolet and γ lesions^{12,13}, may well use common enzymes. As is evident in Fig. 3, normal CRL 1141 and mutant AT3BI cells executed excision repair of ultraviolet photoproducts with identical proficiencies, assayed as either the rate of removal of ultraviolet endonuclease-susceptible sites (that is, pyrimidine dimers¹⁴) or the level of ultraviolet-stimulated repair replication. The other two mutant strains, CRL 1312

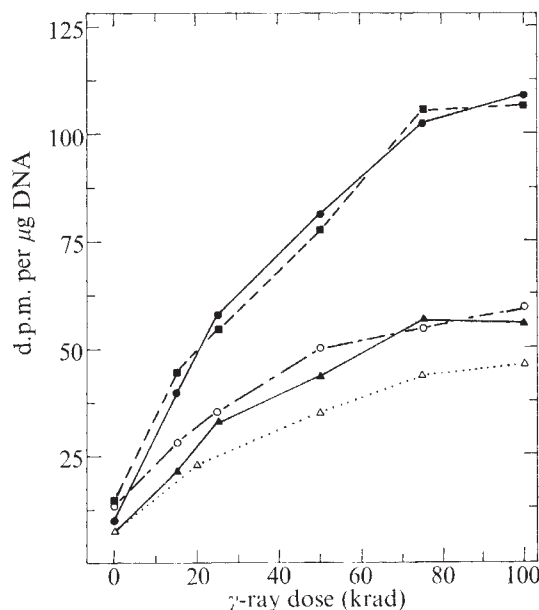


Fig. 2 Dose-dependent levels of repair replication induced in the DNA of normal (●, CRL 1141; ■, CRL 1147) and AT (○, AT3BI; ▲, CRL 1343; △, CRL 1312) strains by ^{60}Co γ -radiation delivered under anoxia. To assess their ability to carry out γ -stimulated repair replication, the human cell lines underwent a standard regimen designed to maximise the incorporation of exogenous nucleotides into DNA at repaired sites while inhibiting *de novo* synthesis¹⁸. Unlabelled cultures containing $2\text{--}4 \times 10^6$ fibroblasts were grown for 2 h in F12 medium containing 65 μM bromodeoxyuridine (BUdR) and 1 μM fluorodeoxyuridine (FUDR), γ -irradiated under nitrogen, and immediately labelled for 2 h with 10 $\mu\text{Ci ml}^{-1}$ ^3H -methylthymidine (specific activity, 45–53 Ci mmol⁻¹; Amersham, Searle) in F12 medium supplemented with 65 μM BUdR, 1 μM FUDR and 1 mM hydroxyurea. Finally, the cultures were incubated for an additional 1 h in F12 medium containing 65 μM BUdR and 1 μM FUDR. The extent of repair replication occurring during the postradiation labelling period was determined by equilibrium centrifugation of the radioactive and density-labelled DNA in NaI gradients. The method followed was basically that developed by Lohman *et al.*¹⁶ except that one rebanding of 'light' parental DNA was normally necessary to ensure its complete physical separation from contaminating 'heavy' DNA arising from semiconservative *de novo* synthesis. After lysis and DNA extraction, neutral NaI gradients (6 ml, containing 0.22 mmol Na_2SO_3 , 10–20 μg extracted human DNA, 0.1 mg ethidium bromide, 4.69 g NaI, adjusted with addition of either Na_2SO_3 or NaI to 1.5400 g cm^{-3} final density) were prepared and centrifuged to equilibrium (33,000 r.p.m. at 20 °C for at least 60 h in a Spinco Type 40 rotor). After collection (~ 20 fractions per gradient) the light DNA was pooled (normally six 300- μl fractions) and dialysed overnight against a 10 mM Tris-0.15 M NaCl (pH 7.5)–10 mM EDTA buffer. NaI gradients containing the dialysed DNA were prepared and centrifuged as above. After fractionation, the distributions of DNA (measured fluorometrically) and radioactivity were ascertained and analysed to calculate the magnitude of repair replication, expressed as d.p.m. per μg light DNA (typically comprising five 300- μl peak fractions). Datum points represent the average of five or more determinations (s.d. $\leq 15\%$).

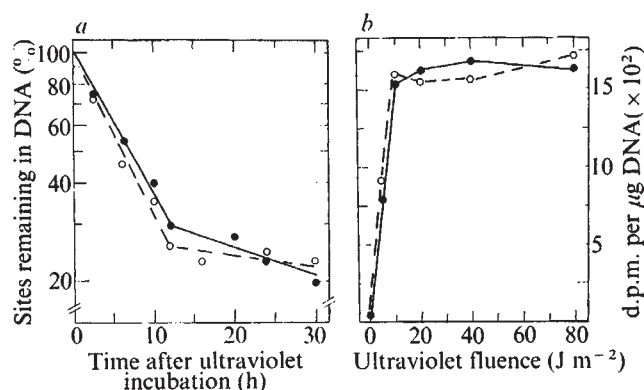


Fig. 3 Excision repair of ultraviolet photoproducts in the DNA of normal (●, CRL 1141) and AT (○, AT3BI) fibroblasts as reflected by *a*, time course of disappearance of ultraviolet endonuclease-susceptible sites; *b*, magnitude of ultraviolet-stimulated repair replication. The fate of nuclease-susceptible sites was monitored with the *in vitro* enzymatic assay as outlined in the caption of Fig. 1 except that: (1) ^3H -labelled cultures were exposed to 5 J m^{-2} 254-nm radiation, and (2) extracted DNA was incubated with highly purified *M. luteus* ultraviolet endonuclease (10 μl per DNA-protein reaction mixture) selectively active toward pyrimidine dimers in ultraviolet-irradiated DNA¹⁴. (The ultraviolet endonuclease was a gift of H. L. Heijneker, Leiden State University, Leiden, The Netherlands.) The amount of repair replication occurring in cultures during 2 h after exposure to specified ultraviolet fluences was determined essentially as described in Fig. 2 except that accurate measurements were obtained without the necessity of rebinding light parental DNA. 100% sites = 2.7 per 10^7 daltons (*a*). Datum points represent the average of triplicate determinations (s.d. $\leq 10\%$).

and CRL 1343, also carried out ultraviolet-induced excision repair with normal proficiency (data to be published elsewhere). It therefore seems that the enzymatic defect in the AT cell lines specifically affects the endonuclease active on γ -modified base residues.

Although the resolution of our assays compelled us to trace the fate of γ radioproducts in moribund cell populations, we are reasonably confident that, within the short postradiation intervals considered here, the metabolic responses assayed portray the actions of *bona fide* repair processes since: (1) the cultures seemed to be normal, both at the cellular and sub-cellular levels; for example, both control and AT strains remained in monolayer with few floating cells and their nuclear DNA was not subject to rampant degradation; (2) control strains, in particular, seemed to achieve considerable success in restoring their DNA to an undamaged stage (Fig. 1).

As observed in *Escherichia coli* minicells¹⁷, the ratio of γ endonuclease-susceptible sites to strand breaks is much greater when the irradiation is delivered to human fibroblasts in anoxic rather than oxic conditions (M.C.P. and B.P.S., unpublished). We therefore maintained cultures in an N_2 atmosphere during irradiation, thereby greatly amplifying the signal-to-noise ratio in both repair assays.

Based on current insight into DNA repair mechanisms^{13,18}, a simple interpretation of our complementary data is that AT cells lack the full complement of functional γ endonuclease and thus fail to initiate excision repair of γ -induced base defects normally. We cannot, however, exclude other less direct explanations including the possibility that the AT strains carry a mutation affecting a later step in the repair process, thereby impeding the normal action of an otherwise unaltered endonuclease activity.

The results of our experiments have important practical and theoretical ramifications, especially in the fields of radiology and molecular radiobiology. Five, in particular, merit brief comment: (1) An inherited deficiency in a specific DNA repair mechanism is indicated as the molecular basis of ataxia telangiectasia. Aside from offering a reasonable and direct explanation for the reported clinical radiosensitivity of AT patients⁶⁻⁸,

our data provide new insight into the cause of this congenital disorder and, although admittedly problematic at present, may ultimately indicate the direction to follow in remedial treatment. (2) AT (ref. 2), like xeroderma pigmentosum¹⁸ (XP), is a congenital disorder in which afflicted individuals are predisposed to an elevated incidence of malignancy, providing additional evidence that defective DNA repair has an aetiological role in at least some forms of neoplastic transformation^{18,24}. (3) AT patients evidently defective in excision repair of radiation-damaged DNA bases are at an adaptive disadvantage; thus, it logically follows that a proficient mechanism to repair these radioproducts is of vital importance to the survival and reproductive capacity of humans. (4) AT strains are proficient in excision repair of ultraviolet photoproducts but defective in excision repair of γ radioproducts, whereas the reverse is found in XP strains¹⁸. These observations are consistent with a working model for this process in which the initial incision step is carried out by different enzymes, γ endonuclease or ultraviolet endonuclease, depending on the type of damage, whereas the remaining steps are executed by common enzymes. A similar model has been proposed for bacterial systems^{22,23}. (5) Considerable success has been achieved in elucidating the excision-repair process active on ultraviolet photoproducts in DNA of human systems¹⁸ after the discovery that XP fibroblasts are defective in ultraviolet-stimulated repair replication²⁴. Since AT seems to be a γ -ray analogue of XP, it might be expected that comparable advances in our understanding of excision repair of γ radioproducts will be forthcoming.

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- 1 Boder, E., and Sedgwick, R. P., *Little Club clin. dev. Med.*, **8**, 110-118 (1963).
- 2 Peterson, R. D., Kelly, W. D., and Good, R. A., *Lancet*, **1**, 1189-1193 (1964).
- 3 Peterson, R. D. A., Cooper, M. D., and Good, R. A., *Am. J. Med.*, **41**, 342-359 (1966).
- 4 Strober, W., Wochner, R. D., Barlow, M. H., McFarlin, D. E., and Waldmann, T. A., *J. clin. Invest.*, **47**, 1905-1915 (1968).
- 5 Harnden, D. G., *Chromosomes and Cancer* (edit. by German, J.), 619-636 (Wiley, New York, 1974).
- 6 Gotoff, S. P., Amirmokri, E., and Liebner, E. J., *Am. J. Dis. Childh.*, **114**, 617-625 (1967).
- 7 Morgan, J. L., Holcomb, T. M., and Morrissey, R. W., *Am. J. Dis. Childh.*, **116**, 557-558 (1968).
- 8 Cunliffe, P. N., Mann, J. R., Cameron, A. H., Roberts, K. D., and Ward, H. W. C., *Br. J. Radiol.*, **48**, 374-376 (1975).
- 9 Higurashi, M., and Conen, P. E., *Cancer*, **32**, 380-383 (1973).
- 10 Taylor, A. M. R., et al., *Nature*, **258**, 427-429 (1975).
- 11 Hoar, D. I., and Sargent, P., *Am. J. hum. Genet.* (in the press).
- 12 Regan, J. D., and Setlow, R. B., *Cancer Res.*, **34**, 3318-3325 (1974).
- 13 Cerutti, P. A., *Life Sci.*, **15**, 1567-1575 (1974).
- 14 Paterson, M. C., Lohman, P. H. M., and Sluyter, M. L., *Mutat. Res.*, **19**, 245-256 (1973).
- 15 Paterson, M. C., *Adv. Radiat. Biol.*, **7**, (in the press).
- 16 Lohman, P. H. M., Sluyter, M. L., Matthijs, I. A. A., and Kleijer, W. J., *Analyt. Biochem.*, **54**, 178-187 (1973).
- 17 Paterson, M. C., and Setlow, R. B., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2927-2931 (1972).
- 18 Cleaver, J. E., *Adv. Radiat. Biol.*, **4**, 1-75 (1974).
- 19 Ham, R. G., *Proc. natn. Acad. Sci. U.S.A.*, **53**, 288-293 (1965).
- 20 Vincent Jr., R. A., Sheridan III, R. B., and Huang, P. C., *Mutat. Res.*, **33**, 357-366 (1975).
- 21 Schneider, E. L., Stanbridge, E. J., and Epstein, C. J., *Expl Cell Res.*, **84**, 311-318 (1974).

²² Strniste, G. F., and Wallace, S. S., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1997–2001 (1975).

²³ Noguti, T., and Kada, T., *Biochim. biophys. Acta*, **395**, 294–305 (1975).

²⁴ Cleaver, J. E., *Nature*, **218**, 652–656 (1968).

²⁵ Carrier, W. L., and Setlow, R. B., *J. Bact.*, **102**, 178–186 (1970).

Sister chromatid exchanges in ageing and repair-deficient human fibroblasts

THE nature of the involvement of DNA repair in the formation of sister chromatid exchanges (SCEs) and their connection with chromosome aberrations is unknown. Some mutagens that damage DNA, including ultraviolet light and mitomycin C, considerably increase the incidence of SCEs^{1–6}, whereas other agents, such as X rays^{6–8}, nitrosoguanidine² and 8-ethoxycaffeine⁵ cause only a slight increase, although they are potent inducers of lesions at the DNA and chromosome levels. Hamster cells infected with adenovirus 12 show no increased SCEs in spite of extensive fragmentation of the entire chromosome complement (our unpublished results). These findings suggest that the mechanism of formation of SCEs is not unitary. Whereas the mechanism of induction of SCEs could be studied by analysing their frequency after application of agents with known action on DNA, spontaneously occurring SCEs can be studied in cells with defects in the DNA repair system. This report deals chiefly with the frequency of spontaneous SCEs in several strains of xeroderma pigmentosum (XP) fibroblasts which show various levels of repair capacities, in Fanconi's anaemia (FA) cells and in control human cells at different stages of ageing.

We used three XP cell strains with different DNA repair capacities^{9–11}, three FA cell strains from unrelated patients and three fibroblast strains from unaffected subjects. All cultures were grown in Eagle's minimal essential medium supplemented with 15% foetal calf serum, and they were used for experiments at their earlier passages. To study SCE, cultures were treated for 38–42 h with 5-bromo-2'-

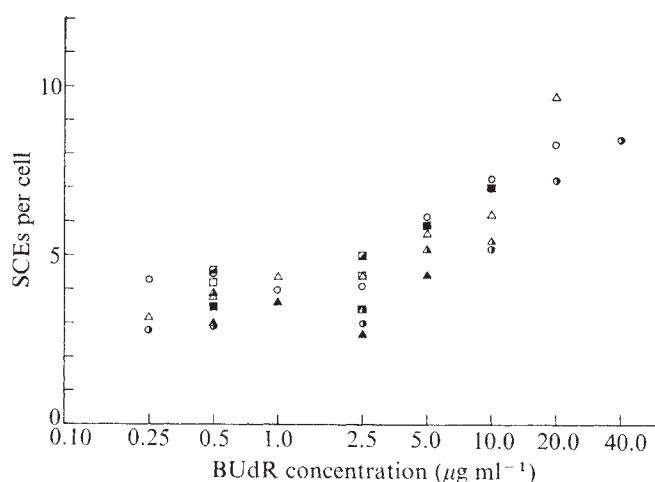


Fig. 1 Effects of BUdR on the frequency of SCEs in various human fibroblast strains. PEG (○) and BRU (half-shaded circle) were control strains established from skin punch biopsies of normal subjects. XP-E (△) and XP-C (half-shaded triangle) were obtained from skin biopsies of two unrelated XP patients and showed 21% and 32%, respectively, of normal unscheduled DNA synthesis after ultraviolet irradiation^{6,8}. XP 1162 (▲) was obtained from the American Type Culture Collection (ATCC). This strain has been reported to have a normal repair capacity as measured by unscheduled uptake of ³H-thymidine after ultraviolet irradiation. FA 122 (□) was an ATCC strain. FA 40400 (half-shaded square) and FA 15100 (■) were gifts from Dr Siminovich University of Toronto. Each point was determined based on 10–25 cells which had been collected at the 2nd metaphase after labelling and stained with acridine orange (0.125 mg ml⁻¹).

Table 1 The frequency of sister chromatid exchanges in chromosomes of various human fibroblasts labelled with BUdR at concentrations lower than 2.5 μg ml⁻¹

Cell strains (sex)	No. of cells observed	No. of SCEs scored	SCEs per cell (± s.e.m.)	Cells with chromosome aberrations† (%)
Normal				
PEG (F)	90	382	4.24 ± 0.22	—
BRU (M)	60	193	3.22 ± 0.23	1.8
HE-1* (F)	350	1,325	3.78 ± 0.10	1.2
Xeroderma pigmentosum				
XP-E (F)	57	249	4.37 ± 0.28	2.9
XP-C (M)	80	289	3.61 ± 0.21	1.5
XP 1162 (M)	30	91	3.03 ± 0.32	0
Fanconi's anaemia				
FA 122 (M)	40	173	4.33 ± 0.33	13.6
FA 40400 (F)	27	130	4.81 ± 0.42	11.0
FA 15100 (M)	50	169	3.38 ± 0.26	15.0

*Embryonic fibroblast strain at passage 12 (a gift from Dr Nakagome of National Institute of Genetics, Mishima). Exact ages of other cultures are uncertain but all were used for experiments within 2–3 passages after thawing of frozen stocks prepared earlier at passages after initiation or purchase of the cultures.

†Determined on 70–200 cells each. There is no statistically significant difference in the aberration frequencies between the normal and XP cells as well as among strains in each group, while the frequencies obtained for all FA cells differ significantly from those in cell strains in the other two groups ($P < 0.01$). Most aberrations detected in FA cells as well as in the other strains were chromatid gaps and breaks.

deoxyuridine (BUdR) at various concentrations and fixed for chromosome preparation after 1 h of treatment with either colchicine or Colcemid. Chromosome preparations were made by the air-drying method and stained with acridine orange². SCEs were scored under fluorescence microscopy.

Figure 1 shows that SCE frequency varied only slightly in each cell strain after treatment with BUdR in concentrations ranging from 0.25 to 2.5 μg ml⁻¹, confirming previous results^{3,4}. We refer to this plateau level as spontaneous SCE although we cannot exclude the possibility that even these exchanges resulted from the application of BUdR¹². To compare the frequencies of SCEs in various cell strains, samples were treated with BUdR at concentrations less than 2.5 μg ml⁻¹. There was no major difference in SCE frequency among fibroblast strains (Table 1). Irrespective of the DNA repair capacity of the cell, SCE frequencies varied between 3 and 4.5 per diploid chromosome complement. It is interesting that FA cells, in spite of high incidence of spontaneous chromosome aberration (Table 1) showed no increased level of sister chromatid exchanges. This observation suggests that, at least in the FA cells examined, the formation of spontaneous chromosome aberrations is dissociated from processes leading to SCE. XP cells seem to have a reduction in the endonuclease activity which is necessary to initiate excision repair¹³. But a class of XP, the XP variant, is characterised by its normal excision repair and suspected to be defective in post-replication repair¹⁴. FA cells are believed to be defective in some DNA repair mechanisms^{15,16}, probably lacking one or more enzymes¹⁷. Our present results are consistent with previous reports on the yield of SCE in XP¹⁸ and FA cells¹⁹. The genesis of spontaneous SCEs may be independent of these particular repair mechanisms.

The spontaneous exchange frequency seems to be affected by the age of cell culture. The normal human fibroblast strain HE-1, at passage 12, was used as a starting culture and grown for about two months; its SCE frequency was examined periodically. To minimise division delay caused by frequent subculturing, we used a 1:6 split scheme at 3 or 4-d intervals during the first month of culture and at

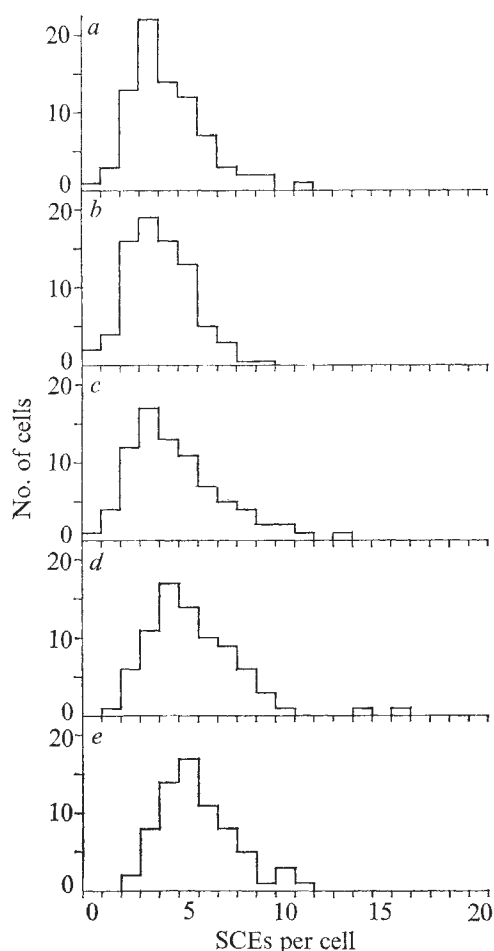


Fig. 2 The shift in SCE frequency during long term culture of HE-1. According to the subculturing scheme used, the culture is considered to undergo about two and a half population doublings per passage. Passages 13(a), 19(b), 24(c), 27(d) and 30(e) may therefore correspond roughly to 33, 48, 60, 68 and 75 population doublings, respectively. Cultures were treated for 40 h with BUdR ($0.25 \mu\text{g ml}^{-1}$) before collection. Mean $\pm$ s.e.: a, 3.99 ± 0.22 ; b, 3.63 ± 0.21 ; c, 4.46 ± 0.24 ; d, 5.28 ± 0.26 ; e, 5.41 ± 0.26 .

longer intervals thereafter. According to this scheme each passage would correspond roughly to 2.5 population doublings. In these conditions, cells ceased growth after passage 32, about 80 d after the start of the experiment. A statistically significant increase in SCE frequency was observed during long term culture but only at the end of the life span of the culture. As Fig. 2 shows the modal value of the exchange frequency per diploid chromosome complement was 3 at the onset of the experiment (at the 33rd population doubling level). This value remained unchanged up to passage 24 (60 doublings), then moved to 4 at passage 27 (68 doublings) and reached 5 at passage 30 (at the 75th population doubling level). At this last period the cell cultures contained many slow growing cells which showed several symptoms of senescence, including considerable variation in size and morphology and an increased level of chromosome aberration. The incidences of cells having chromosome breaks and exchanges were 1.2%, 2.1%, 5.3% and 9.6% at passages 13, 24, 27 and 30, respectively. This increased production of aberrations during the latest part of the life span seems to be compatible with the finding of Saksela and Moorhead's findings²⁰ with WI-38 cells, and parallels the increase in the SCE frequency.

The results obtained with XP and FA cells obtained in this study support the conclusion¹⁸ that the induction of

spontaneous SCE cannot be explained by a simple application of concepts from known DNA repair mechanisms. On the other hand, the observed link between SCE and chromosome aberrations in the ageing cell culture and in Bloom's syndrome¹⁹ and their dissociation in FA cells indicate that the pathways through which both events arise are at least partially different.

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- Rommelaere, J., Suskind, M., and Errera, M., *Chromosoma*, **41**, 243-257 (1973).
- Kato, H., *Expl Cell Res.*, **82**, 383-390 (1973); **85**, 239-247 (1974); **89**, 416-420 (1974); *Nature*, **249**, 552-553 (1974); **251**, 70-72 (1974).
- Kato, H., and Shimada, H., *Mutat. Res.*, **28**, 459-464 (1975).
- Latt, S. A., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3162-3166 (1974).
- Kihlman, B. A., *Chromosoma*, **51**, 11-18 (1975).
- Perry, P., and Evans, H. J., *Nature*, **258**, 121-125 (1975).
- Marin, G., and Prescott, D. M., *J. Cell Biol.*, **21**, 159-167 (1964).
- Gatti, M., and Olivieri, G., *Mutat. Res.*, **17**, 101-112 (1973).
- Stich, H. F., and San, R. H. C., *Mutat. Res.*, **13**, 279-282 (1971).
- Stich, H. F., San, R. H. C., Miller, J. A., and Miller, E. C., *Nature new Biol.*, **238**, 9-10 (1972).
- Stich, H. F., Stich, W., and Lam, P., *Nature*, **250**, 599-601 (1974).
- Wolff, S., and Perry, P., *Chromosoma*, **48**, 341-353 (1974).
- Setlow, R. B., Regan, J. D., German, J., and Carrier, W. L., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 1035-1041 (1969).
- Lehmann, A. R., et al., *Proc. natn. Acad. U.S.A.*, **72**, 219-223 (1975).
- Sasaki, M. S., *Nature*, **257**, 501-503 (1975).
- Fujiwara, Y., and Tatsumi, M., *Biochem. biophys. Res. Commun.*, **66**, 592-598 (1975).
- Poon, P. K., O'Brien, R. O., and Parker, J. W., *Nature*, **250**, 223-225 (1974).
- Wolff, S., Bodycote, J., Thomas, G. H., and Cleaver, J. E., *Genetics*, **81**, 349-355 (1975).
- Chaganti, R. S. K., Schonberg, S., and German, J., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4508-4512 (1974).
- Saksela, E., and Moorhead, P. S., *Proc. natn. Acad. Sci. U.S.A.*, **50**, 390-395 (1963).

Differential chromatid staining by *in vivo* treatment as a mutagenicity test system

DIFFERENTIAL staining of sister chromatids can be demonstrated with Hoechst 33258 fluorochrome¹ and with Giemsa after special pretreatment², even if 5-bromodeoxyuridine (BUdR) is present during the first S phase only³. These techniques permit the use of the phenomenon of the sister chromatid exchange (SCE). The average spontaneous rate of 10-14 SCEs per metaphase seems to be fairly constant in all cultures from several species^{4,5}. A variety of mutagenic factors (that is, ultraviolet light and alkylating agents) produce very high frequencies of SCE even at concentrations far below the level necessary for the induction of chromosomal breakage, whereas others, like X rays or certain chemicals, hardly influence the rate of SCE⁶. Because of the high turnover of BrdU in the liver and the physiologically available thymidine, differential chromatid staining has so far only been produced in *in vitro* systems (cell cultures) and in the chicken embryo.

We have found that the additional application of 5-fluorodeoxyuridine (FUDR) which inhibits thymidine synthesis leads to differential chromatid staining in metaphases prepared directly from bone marrow. This method proved to be a sensitive test system for mutagenicity as demonstrated by the increasing frequencies of SCE after application of two alkylating agents.

Mature mice were injected with 0.4 mg FUDR per g body weight intraperitoneally; beginning 3 h later they received

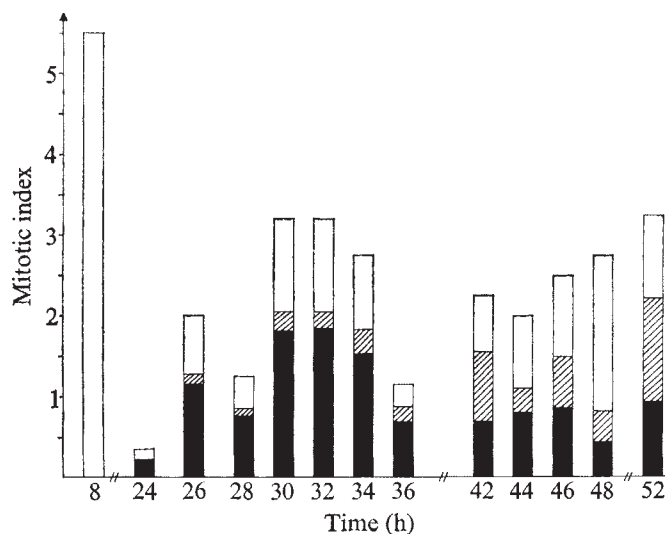


Fig. 1 Solid column, second mitoses (complete differential chromatid staining); hatched column, third mitoses.

six times 0.04 mg BUdR per g body weight during 6 h. Colcemid (16 µg) was administered 3 h before killing. Metaphases from bone marrow were prepared using standard methods without culturing, and stained according to Epplen *et al.*⁸. Mitotic indices and frequencies of differential chromatid staining in the second and the following mitoses are given in Fig. 1. The highest incidence of second mitoses appears about 30 h after the first BUdR injection. About 2 h after BUdR treatment a replication pattern can be found.

Thus a method has been developed with the capacity to induce differential chromatid staining by *in vivo* treatment. Compared with *in vitro* studies the 'spontaneous' rate of SCE turned out to be extremely low (4 per metaphase). Possible explanations for this difference may include, for example, the complete absence of light from the cells in the bone marrow or a lower mean concentration of BUdR during treatment. To demonstrate the efficiency of the method for mutagenicity testing we have studied the induction of SCE by two alkylating agents *in vivo*. Both substances produce a dose-dependent increase in SCEs (Table 1) which can be detected at concentrations below those inducing chromosomal breakage (Fig. 2). Using higher

Table 1 SCE per metaphase after administration of various concentrations of triaziquon and cyclophosphamide

µg per g body weight	SCE per metaphase
Triaziquon	
0	4
0.0025	6
0.0125	10
0.125	30
Cyclophosphamide	
0	4
12.5	15
25	24

Each value represents at least 20 metaphases of two different specimens.

concentrations, the mitotic index and the proportion of differentially stained metaphases is greatly reduced by the toxic activity of the drugs. As to be expected, the effect of cyclophosphamide is evident in our *in vivo* system, whereas it was inert in *in vitro* studies⁹. In so far as the induction of SCE can be regarded as an indication for mutagenicity⁹, our method enables the application of this test system to *in vivo* studies.

Since *in vitro* systems of mutagenicity testing are subject to methodical errors, the transfer of these systems into physiological conditions, that is, *in vivo*, is preferable. If the restrictions with respect to the significance of SCE are borne in mind⁹, our method may turn out to be a useful tool in mutagenicity testing.

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- 1 Latt, S., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3395-3399 (1973).
- 2 Perry, P., and Wolff, S., *Nature*, **251**, 156-158 (1974).
- 3 Korenberg, J. R., and Freedlender, E. F., *Chromosoma*, **48**, 355-360 (1974).
- 4 Wolff, S., and Perry, P., *Chromosoma*, **48**, 341-353 (1974).
- 5 Galloway, S. M., and Evans, H. J., *Cytogenet. Cell Genet.*, **15**, 17-29 (1975).
- 6 Perry, P., and Evans, H. J., *Nature*, **258**, 121-125 (1975).
- 7 Bloom, S. E., and Hsu, T. C., *Chromosoma*, **51**, 261-267 (1975).
- 8 Epplen, J. T., Siebers, J.-W., and Vogel, W., *Cytogenet. Cell Genet.*, **15**, 177-185 (1975).
- 9 Savage, K. R., *Nature*, **258**, 103-104 (1975).

Fig. 2 Bone marrow metaphase with differential chromatid staining showing a high incidence of SCE after *in vivo* treatment of the mouse with cyclophosphamide.



Analysis of sister chromatid exchange formation *in vivo* in mouse spermatogonia as a new test system for environmental mutagens

SPERMATOGONIA, as stem cells of male gametes^{1,2}, constitute a highly relevant system for studying the genetic hazards of potential mutagens. Gross chromosome aberrations in spermatogonia have been used as a gauge of the genetic damage induced in animals exposed to agents such as mitomycin C (refs 3-5). Sister chromatid exchanges (SCEs) formed in response to DNA damage constitute even more sensitive indices of the impact of alkylating agents and other clastogens on chromosomes⁶⁻⁸. Analysis of SCE induction in cultured cells has been greatly facilitated by techniques in which substitution of DNA with the base analogue 5-bromodeoxyuridine (BUdR) is detected either with fluorescent dyes^{9,10} or by modified Giemsa procedures¹¹⁻¹³. This approach has so far been limited to *in vitro* trials, with the exception of one system¹⁴ in which SCEs were visualised in chromosomes of chick embryos after exposure to BUdR *in ovo*. We report here a BUdR technique which enables the detection of SCEs formed in spermatogonial cells of intact mice. As a prototype of a

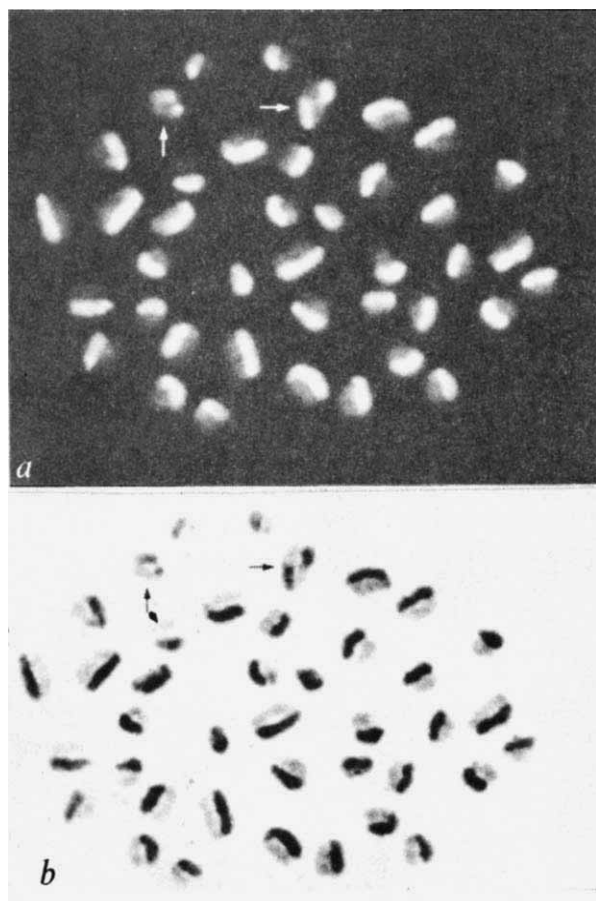


Fig. 1 Sister chromatid differentiation and SCEs in mouse spermatogonia. After *in vivo* administration of BUdR and slide preparation as described in the text, this cell was stained with 33258 Hoechst. *a*, Result of fluorescence microscopy; *b*, appearance of the same cell following subsequent Giemsa staining. SCEs indicated by arrows.

general *in vivo* mutagenesis test, this procedure has been utilised to demonstrate a several-fold increase in the sister chromatid exchange frequency in mouse spermatogonia after injection of the animals with small amounts of mitomycin C.

Substitution of spermatogonial chromatids in 3–5-month-old male CBA/J mice was effected by administering 14 hourly intraperitoneal (0.2 ml) injections of BUdR (10^{-2} M) together with deoxycytidine (5×10^{-3} M), the latter to reduce BUdR toxicity¹⁵. The series of injections served to replenish exogenous nucleotide which is thought to undergo rapid depletion *in vivo*¹⁶. The total duration of exposure to BUdR (approximately 14 h) is presumed sufficient for incorporation of this base analogue into all spermatogonial

subpopulations, including the predominating type B spermatogonia which exhibit the longest DNA synthesis periods¹⁷. Mitomycin C, when administered, was injected as a single intraperitoneal dose (0.025 – 0.5 mg kg⁻¹) 26 h after the initial injection of BUdR. After an additional 28-h period, Colcemid was injected (0.6 mg kg⁻¹, intraperitoneally) and the animals were killed by ether narcosis 3–5 h later. Spermatogonial chromosomes were prepared from isolated seminiferous tubules¹⁸, stained with 33258 Hoechst^{9,19}, and sister chromatid differentiation examined by fluorescence microscopy which, in many cases, was followed by Giemsa staining^{11,12}.

Most of the metaphase cells examined revealed the differential chromatid fluorescence characteristic of 33258 Hoechst stained cells which had replicated twice after administration of BUdR²⁰ (Fig. 1). Similar patterns could be observed with Giemsa staining after the intense illumination used for fluorescence microscopy¹². In view of the schedule of BUdR administration and the generation times of the spermatogonial cells¹⁷, the present sister chromatid differentiation is believed to reflect one cycle of BUdR

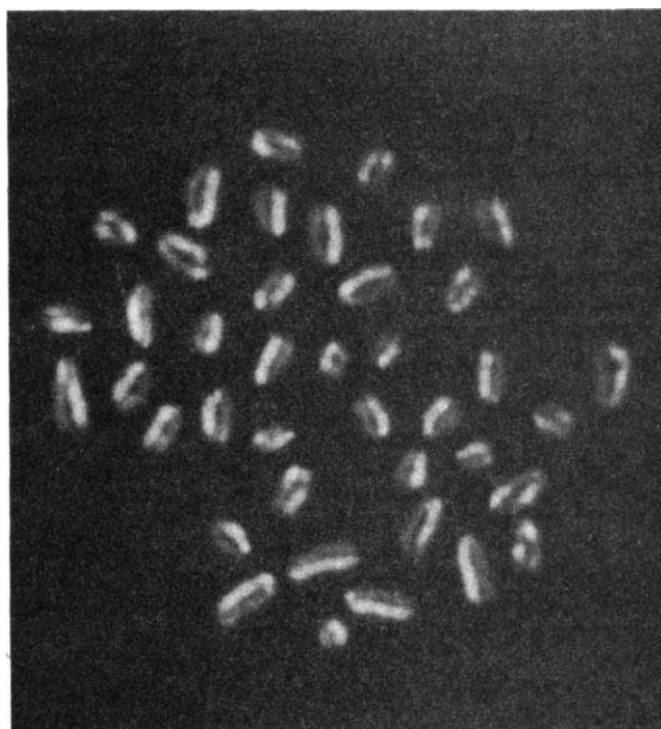


Fig. 2 Extensive induction of SCEs in a mouse spermatogonial cell by mitomycin C. This cell was treated as described in Fig. 1, except that a single dose of mitomycin C (0.5 mg kg⁻¹) was injected intraperitoneally into the animal 32 h before collection. Eighteen SCEs are evident in this cell.

Table 1 SCEs and chromosome aberrations induced by mitomycin C in BUdR-substituted spermatogonia

Mitomycin C dosage (mg kg ⁻¹)	Mice	Metaphases†	Genomes‡	SCEs	SCEs per genome	Aberrations§ per genome
0 (control)	4	202	261	473	1.81	0.01
H ₂ O (control)	2	98	121	200	1.65	0.02
0.025	3	77	95	199	2.09	0.00
0.05	4	125	156	562	3.60	0.04
0.10	4	114	140	506	3.62	0.03
0.30	4	132	179	863	4.82	0.03
0.50	3	78	99	716	7.23	0.02

*Injections (approximately 0.3 ml) were given approximately 31–33 h before cell collection.

†Diploid- and polyploid-appearing cells.

‡A diploid genome contained 40 chromosomes.

§Aberrations included translocations, chromatid breaks and quadriradial figures.

incorporation followed by a second cycle in the relative absence of analogue. Metaphase cells collected at earlier times (23 h after initiation of BUdR administration) and stained with 33258 Hoechst demonstrated the lateral fluorescence asymmetry expected for satellite DNA containing centromeric regions which have undergone one round of BUdR incorporation²¹. An occasional second division cell exhibited a longitudinal fluorescence differentiation, reflecting BUdR incorporation for only part of a DNA synthesis period, thereby suggesting usefulness of this method for studying replication kinetics.

An average of somewhat less than 2 SCEs per cell were observed for control animals given BUdR but not mitomycin C (Table 1). Frequencies are normalised for the numerous multiple genome figures reflecting the interconnected cell populations characteristic of male germinal tissue²². The SCE rate increased to two, three and four times that of control at mitomycin C doses of 0.05, 0.3, and 0.5 mg kg⁻¹, respectively. The last dose, which markedly increased the exchange frequency (Fig. 2), induced fewer than 0.1 morphological aberrations (breaks, rearrangements) per second division metaphase. Consistent with other reports^{3,23}, higher doses of mitomycin C (1.0–5.0 mg kg⁻¹) markedly reduced the number of metaphase cells available for analysis, presumably by retarding the proliferation of severely damaged spermatogonia. Preliminary observations using the techniques described above indicate that intraperitoneal injection of mitomycin C is also capable of inducing SCEs in mouse bone marrow cells.

The use of intact animals in the present study provides a highly relevant test system which takes into account host metabolism and possible activation of the drug^{24,25}, as well as passage of the drug across the blood–testis barrier²⁶. Technical improvements in this procedure, in particular those simplifying the administration of BUdR to animals, should facilitate application of this approach. The precise relationship between SCE induction and mutagenesis remains to be determined, although the two events exhibit a high correlation⁸. Mitomycin C-induced chromosome rearrangements in spermatogonia have already been implicated in mutagenesis^{4,27} and errors potentially associated with SCE formation may similarly contribute to the mutation frequency²⁸. Thus, SCE analysis in mammalian spermatogonia should constitute an important new test of environmental mutagenesis.

An additional potential of a spermatogonia cell system for BUdR-dependent differentiation between sister chromatids is its natural extension for experimental studies of meiosis. If collection of the seminiferous tubules is delayed for approximately 2 weeks after BUdR administration, BUdR and therefore sister chromatid differentiation might be present in condensed meiotic chromosomes²⁹. Optical detection of chromatid exchange between homologues should then enable high resolution analysis of meiotic recombination, which could also be compared with SCE frequencies in different cell types of the same animals.

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- ¹ Oakberg, E. F., *Mutat. Res.*, **11**, 1–7 (1971).
- ² Clermont, Y., *Physiol. Rev.*, **52**, 198–236 (1972).
- ³ Manyak, A., and Schleiermacher, E., *Mutat. Res.*, **19**, 99–108 (1973).
- ⁴ Adler, I., *Mutat. Res.*, **23**, 369–379 (1974).
- ⁵ Bempong, M. A., and Trower, E. C., *J. Hered.*, **66**, 285–289 (1975).
- ⁶ Kato, H., *Expl Cell Res.*, **85**, 239–247 (1974).
- ⁷ Latt, S. A., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3162–3166 (1974).
- ⁸ Perry, P., and Evans, H. J., *Nature*, **258**, 121–125 (1975).
- ⁹ Latt, S. A., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3395–3399 (1973).

- ¹⁰ Kato, H., *Nature*, **251**, 70–72 (1974).
- ¹¹ Perry, P., and Wolff, S., *Nature*, **251**, 156–158 (1974).
- ¹² Kim, M. A., *Humangenetik*, **25**, 179–188 (1974).
- ¹³ Korenberg, J., and Freedlander, E., *Chromosoma*, **48**, 355–360 (1974).
- ¹⁴ Bloom, S. E., and Hsu, T. C., *Chromosoma*, **51**, 261–267 (1975).
- ¹⁵ Meuth, M., and Green, H., *Cell*, **2**, 109–112 (1974).
- ¹⁶ Russev, G., and Tsanev, R., *Methods in Cell Biology*, IX (edit. by Prescott, D. M.), ch. 8 (Academic, New York, 1975).
- ¹⁷ Monesi, V., *J. Cell Biol.*, **14**, 1–18 (1962).
- ¹⁸ Meredith, R., *Chromosoma*, **26**, 254–258 (1969).
- ¹⁹ Latt, S. A., *J. Histochem. Cytochem.*, **22**, 478–491 (1974).
- ²⁰ Latt, S. A., *Science*, **185**, 74–76 (1974).
- ²¹ Lin, M. S., and Davidson, R. L., *Science*, **185**, 1179–1181 (1974).
- ²² Dym, M., and Fawcett, D. W., *Biol. Reprod.*, **4**, 195–215 (1971).
- ²³ Gilliavod, N., and Leonard, A., *Mutat. Res.*, **13**, 274–275 (1971).
- ²⁴ Legator, M. S., and Malling, H. V., *Chemical Mutagens*, 2 (edit. by Hollaender, A.), ch. 22 (Plenum, New York, 1971).
- ²⁵ Ames, B. N., McCann, J., and Yamasaki, E., *Mutat. Res.*, **31**, 347–364 (1975).
- ²⁶ Vitale, R., Fawcett, D. W., and Dym, M., *Anat. Res.*, **176**, 333–344 (1973).
- ²⁷ Ehling, U. H., *Mutat. Res.*, **26**, 285–295 (1974).
- ²⁸ Heddle, J. A., and Bodycote, D. J., *Mutat. Res.*, **9**, 117–126 (1970).
- ²⁹ Kofman-Alfaro, S., and Chandley, A. C., *Chromosoma*, **31**, 404–420 (1970).

Fate of thiazolidine ring during fragmentation of penicillin by exocellular DD-carboxypeptidase-transpeptidase of *Streptomyces* R61

LIKE various β -lactamases, acylases and esterases¹, the exocellular DD-carboxypeptidase-transpeptidase of *Streptomyces* R61 degrades benzylpenicillin and other β -lactam antibiotics^{2–4}. The R61 enzyme, however, markedly differs from the other penicillin-degrading enzymes in causing fragmentation of the penicillin nucleus. By using 8-¹⁴C-benzylpenicillin (benzyl labelled) as substrate, one of the fragments produced was shown to be ¹⁴C-phenylacetyl-glycine⁵. The reaction with the R61 enzyme is also peculiar in that it is a slow process. This is because of the long half life of the stoichiometric complex transiently formed between the antibiotic and the enzyme. Thus, for example, the value of the half life for the complex formed with benzylpenicillin is 80 min in 10 mM phosphate buffer (pH 7.0) and at 37 °C. As breakdown of the complex proceeds, however, phenylacetyl-glycine (when benzylpenicillin is used as substrate) is released and the enzyme concomitantly recovers its ability to bind penicillin. We have now characterised the fragment (hereby designated as the Y product) arising from the thiazolidine ring of penicillin as a result of the fragmentation of the antibiotic molecule by the R61 enzyme.

The large amount (about 20 μ mol) of ¹⁴C-phenylacetyl-glycine previously produced from 8-¹⁴C-benzylpenicillin through a series of enzyme inactivation and reactivation (total time for the operation, 100 h) could be purified by filtration of the reaction mixture on a column of Sephadex G-25 on the basis that phenylacetyl-glycine was eluted at a volume slightly larger than the salt volume⁵. Examination of the fractions containing ¹⁴C-phenylacetyl-glycine by NMR spectrometry failed to reveal any lines that could be attributed to methyl groups. In marked contrast, the NMR spectrum in D₂O of the non-radioactive material obtained from the salt-containing fractions exhibited lines characteristic of methyl groups. The spectrum indicated that the thiazolidine ring had been opened and suggested that the split product was either *N*-formyl-D-penicillamine or di-(*N*-formyl-D-penicillamine)disulphide (Fig. 1). Direct chromatography of a sample of this fraction on Aminex A5 ion-exchange resin yielded only very minor ninhydrin-positive peaks. Mild acid hydrolysis (1 h in 1 N HCl at 60 °C, that is, in conditions which are known to release the formyl group from di-(*N*-formyl-D-penicillamine)disulphide⁶) produced one important ninhydrin-positive compound which had the same behaviour on the Aminex column as di-(D-penicillamine)-disulphide. Finally, mild acid hydrolysis followed by performic oxidation yielded a highly acidic ninhydrin-positive product which, on the same Aminex column, was indistinguishable from the product obtained by performic oxidation of D-penicillamine.

In order to characterise the Y product further and to study the kinetics of its release, various radioactive penicillins with the radioactive label in the thiazolidine portion of the molecule were prepared. ³H-Benzylpenicillin (labelled in the β -methyl

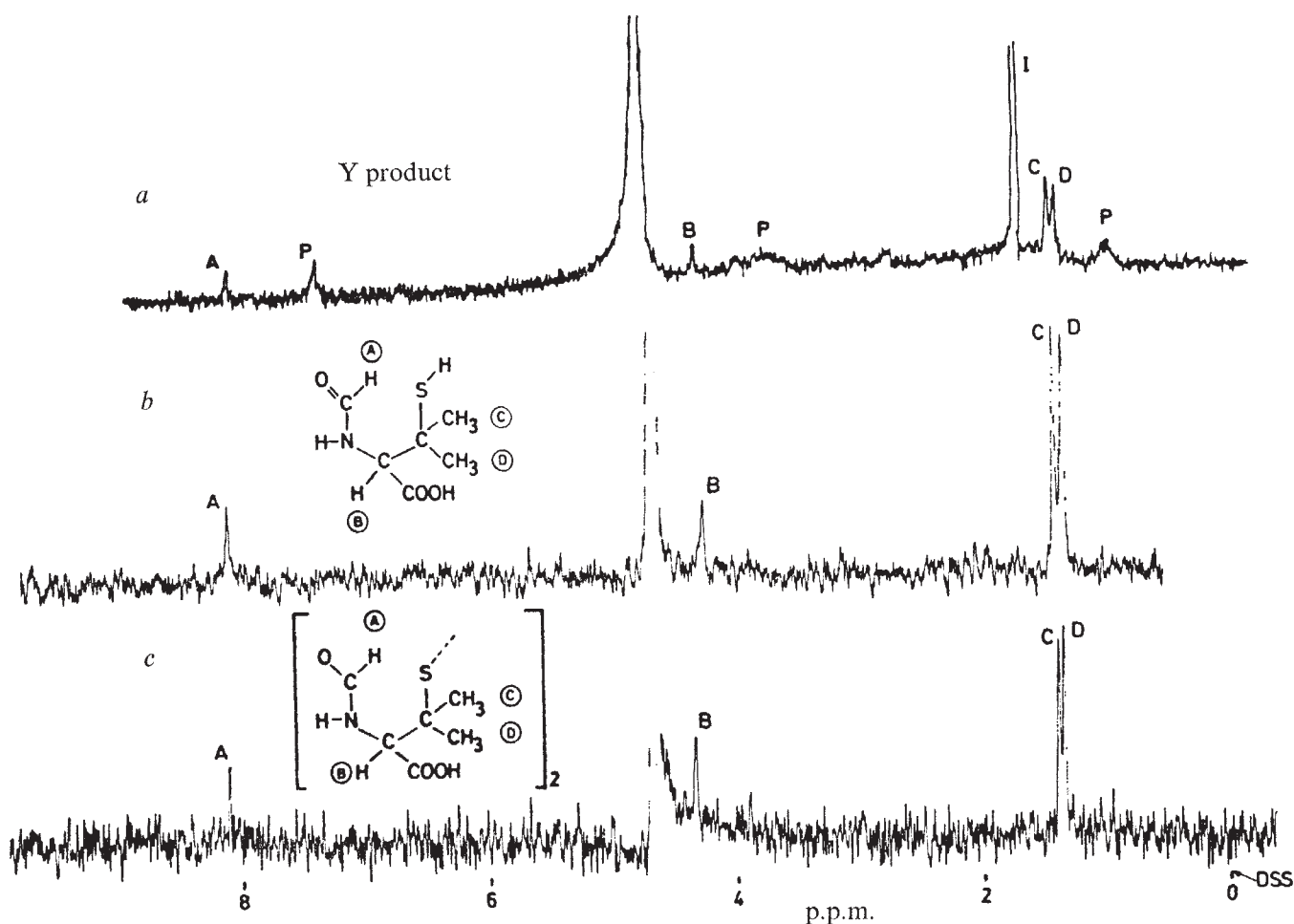


Fig. 1 NMR spectra in D₂O of Y product (a), *N*-formyl-D-penicillamine (b) and di-(*N*-formyl-D-penicillamine)disulphide (c). The spectra were obtained with a Varian XL100-15 spectrometer operated in the ²D internal lock mode. The I line exhibited by the Y preparation (pH 7.0) was attributed to an impurity and the P lines were attributed to the presence of a small amount of penicilloic acid. The A, B, C and D lines, occurring in the ratios of 1:1:3:3, were attributed to the Y product. The 6 Hz distance between the methyl lines (C and D) excluded the presence of an intact thiazolidine ring (in which case the distance value should have been 38 Hz). The spectra of the Y product, *N*-formyl-D-penicillamine and di-(*N*-formyl-D-penicillamine)disulphide were virtually superimposable with respect to lines A (8.09, 8.11 and 8.09 p.p.m., respectively), B (4.31, 4.28 and 4.34 p.p.m., respectively), C (1.40, 1.38 and 1.36 p.p.m., respectively) and D (1.46, 1.45 and 1.41 p.p.m., respectively). The p.p.m. values are relative to 2,2-dimethyl-2-silapentane-5-sulphonate (DSS). Further confirmation was obtained by extracting the Y product (after acidification) with ethylacetate. The extract was evaporated to dryness and the residue dissolved in CDCl₃ in the presence of dimethylsulphoxide. Proton B of the CH group located in the vicinity of the NH group gives a characteristic doublet (9 Hz splitting). The SH proton, however, could not be seen because of the presence of a large impurity peak in its resonance region and it was therefore impossible to decide whether the Y product was *N*-formyl-D-penicillamine or di-(*N*-formyl-D-penicillamine)disulphide. The *N*-formyl-D-penicillamine used as standard (spectrum b) was prepared by dissolving D-penicillamine HCl (550 mg) in 7 ml formic acid (95%) containing 200 mg of sodium formate. After addition of 2 ml of acetic anhydride, the reaction proceeded for 1 h at room temperature. Melting point 146–148 °C; (α _D²⁰ + 63 (C = 1, pyridine)). The optical rotation is in agreement with the value published previously⁶. The di-(*N*-formyl-D-penicillamine)disulphide (spectrum c) was prepared from *N*-formyl-D-penicillamine by oxidation in air in the presence of FeCl₆.

group, 1.7 mCi mmol⁻¹), 5-¹⁴C-benzylpenicillin (0.07 mCi mmol⁻¹) and 5-¹⁴C-phenoxyethylpenicillin (that is, penicillin V; 0.08 mCi mmol⁻¹). As determined by the bioassay and iodometric assay, the purity of the compounds was higher than 98%. Thin-layer chromatography showed, besides the penicillin spot, a small amount of penicilloic acid ($\pm 2\%$) but no other radioactive impurities.

In a first series of experiments, maximal amounts of ³H-Y and ¹⁴C-Y products were prepared from ³H-benzylpenicillin and 5-¹⁴C-benzylpenicillin, respectively. In these assays, the R61 enzyme was maintained saturated by successive additions of radioactive penicillins to the reaction mixtures. The total period of time for the whole operations was 24 h and a total amount of 0.5 μ mol of each radioactive benzylpenicillin was converted into degradation products. The accumulated ³H-Y and ¹⁴C-Y products were partially purified by filtration on Sephadex G-25. The following observations were made. (1) Both radioactive Y products had the same *R_f* values by thin-layer chromatography and with the two solvents used, these *R_f*

values were identical to those of di-(*N*-formyl-D-penicillamine)-disulphide (Table 1). (2) At least 95% of the radioactive labels originally engaged in the reactions, cochromatographed on the Aminex A5 column exactly with exogenously added, non-radioactive di-(*N*-formyl-D-penicillamine)disulphide (as revealed by ninhydrin after acid hydrolysis of a sample of the fractions obtained; Fig. 2a). (3) Treatment of the ³H-Y product with 1 N HCl for 60 min at 60 °C produced (95% yield) a radioactive compound which on the Aminex column cochromatographed exactly with exogenously added, non-radioactive di-(D-penicillamine)disulphide (as revealed directly with ninhydrin; Fig. 2b). (4) HCl Treatment of the ¹⁴C-Y product in the same conditions as above yielded a non-radioactive compound which also behaved on the Aminex column as di-(D-penicillamine)disulphide (and was directly revealed with ninhydrin). Since the ¹⁴C-Y product was formed by action of the R61 enzyme on 5-¹⁴C-benzylpenicillin and since treatment of the ¹⁴C-Y product under conditions known to release the formyl group from di-(*N*-formyl-D-penicillamine)disulphide

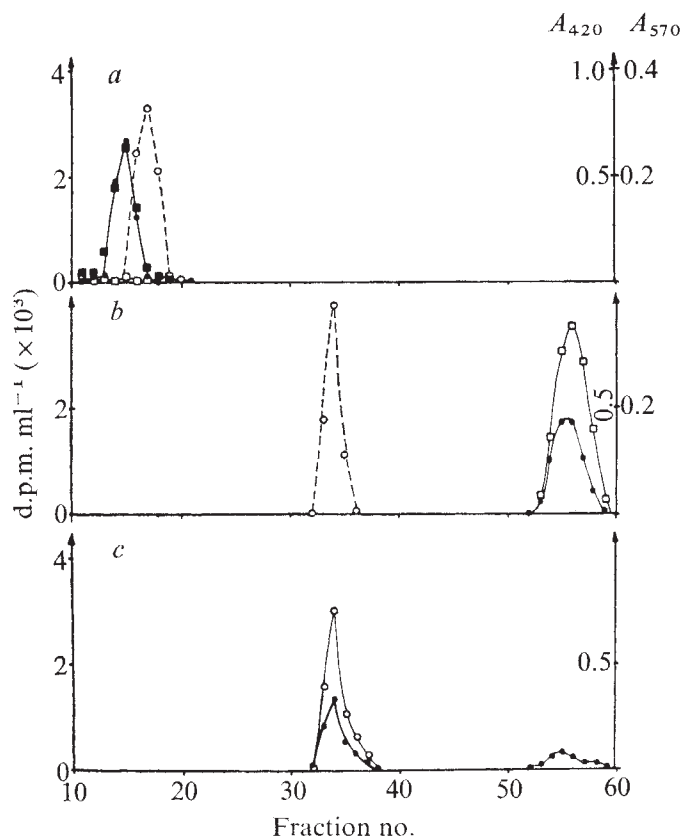


Fig. 2 Identification by chromatography on Aminex A5 of the Y products released from benzylpenicillin after interaction with the R61 enzyme under prolonged (*a* and *b*) and short (*c*) incubation times. The column (9×540 mm) was equilibrated against 0.2 M sodium citrate-HCl buffer (pH 3.17). Elution was performed with the same buffer at a flow rate of 80 ml h⁻¹ (volume of the fractions: 3.2 ml). *a*, A mixture containing 10 nCi of ³H-Y product and 200 µg of each *N*-formyl-D-penicillamine and di-(*N*-formyl-D-penicillamine)disulphide was deposited. Radioactivity (●) was measured on 1-ml samples. The pH of 0.4-ml samples was adjusted to 7.5–8.0 by addition of 40 µl of 2 M Tris base and the free sulphhydryl groups (○) were estimated by adding 20 µl of 50 mM Tris-HCl buffer (pH 8.0) containing 40 µg of 5,5'-dithio-bis(2-nitrobenzoic acid) (*A*₄₂₀). The free amino groups were estimated by reaction with ninhydrin (*A*₅₇₀) before (□) and after (■) a 60 min incubation at 60 °C in 1 N HCl. *b*, A mixture containing 10 nCi of ³H-Y product and 400 µg of di-(*N*-formyl-D-penicillamine)disulphide was incubated at 60 °C for 60 min in 1 N HCl. The pH was then adjusted to 2.2, 200 µg of D-penicillamine were added and the mixture was deposited on the column. Radioactivity (●) was measured on 1-ml samples. Free sulphhydryl groups (○) and free amino groups (□) were estimated on 0.4-ml and 0.5-ml samples, respectively, as in *a*. *c*, Chromatography of the HCl-hydrolysed tritiated compound prepared by a 2-h incubation of ³H-benzylpenicillin with the R61 enzyme (for conditions, see text). The radioactivity (●) was measured on 1-ml samples. Free sulphhydryl groups (○) were estimated as above. The *A*₅₇₀ value after colour development of D-penicillamine with ninhydrin is about 7% of that obtained with equivalent amount of D-penicillamine disulphide.

actually resulted in the release of the radioactive label, it seems clear that the formyl group of di-(*N*-formyl-D-penicillamine)-disulphide arises from C5 of the penicillin nucleus. (5) Thin-layer chromatography in solvent B (for composition, see Table 1) of the hydrolysed ³H-Y and ¹⁴C-Y products revealed the presence of a radioactive ninhydrin-positive spot and of a non-radioactive ninhydrin-positive spot, respectively, exhibiting the same *R*_f value (0.27) as di-(D-penicillamine)disulphide. (6) Finally, performic oxidation of the HCl-treated ³H-Y product yielded a very acidic ³H compound (90% yield) which, on the Aminex column, cochromatographed exactly with exogenously added penicillamine previously oxidised with performic acid.

In a second series of experiments, a half life of 90 min for the ³H-benzylpenicillin-R61 complex (in 10 mM phosphate buffer (pH 7.0) and at 37 °C) was determined on the basis of the release of the radioactive label. In previous studies³, an average half life of 80 min had been determined for the complex formed with the R61 enzyme. Within the limits of experimental errors therefore, recovery of enzyme activity and release of the two fragments arising from the benzylpenicillin molecule occur with the same rate constant ($1.4 \times 10^{-4} \text{ s}^{-1}$). This result together with the facts that after complete enzyme recovery, more than 90% of the original radioactive labels were recovered as ¹⁴C-phenylacetyl-glycine and either ³H- or ¹⁴C-di-(*N*-formyl-D-penicillamine)disulphide (depending on the radioactive benzylpenicillins used), show clearly that phenylacetyl-glycine and di-(*N*-formyl-D-penicillamine) are produced in stoichiometric amounts.

Since the Y product characterised as di-(*N*-formyl-D-penicillamine)disulphide was isolated after several cycles of enzyme inactivation and reactivation (that is, after prolonged incubation), we wondered whether it was actually the one initially released through breakdown of the penicillin molecule. In a third series of experiments, two samples, each containing 5 nmol of ³H-benzylpenicillin and 6 nmol of R61 enzyme, were incubated for 2 h at 37 °C under nitrogen. Reaction of one of the samples with 5,5'-dithio-bis(2-nitrobenzoic acid) indicated free SH groups. Non-radioactive *N*-formyl-D-penicillamine (125 µg) was added to the second sample and the solution was treated with 1.2 N HCl (final concentration) for 16 h at 60 °C, that is in conditions where release of the formyl group from *N*-formyl-D-penicillamine was known to occur⁶. Chromatography on Aminex A5 revealed that 80% of the radioactive product present in the hydrolysate was penicillamine (Fig. 2c). About 20% of penicillamine disulphide was detected. Thus *N*-formyl-D-penicillamine was probably the primary product released and di-(*N*-formyl-D-penicillamine)disulphide was a product subsequently formed by oxidation. Surprisingly, however, by incubating 50 nmol of *N*-formyl-D-penicillamine for 16 h at 37 °C, in 100 µl of 10 mM phosphate buffer (pH 7.0),

Table 1 Thin-layer chromatography on silica gel (G) of radioactive Y product and of non-radioactive *N*-formyl-D-penicillamine and di-(*N*-formyl-D-penicillamine)disulphide

Compound	<i>R</i> _f values in solvents	
	A	B
³ H- or ¹⁴ C-Y product	0	0.69
<i>N</i> -formyl-D-penicillamine	0.34	0.68
di-(<i>N</i> -formyl-D-penicillamine)disulphide	0	0.67

Solvent A, CHCl₃/CH₃OH/CH₃COOH: 88/10/2 (v/v/v).

Solvent B, 1-butanol/H₂O/C₂H₅OH/CH₃COOH: 10/4/3/3 (v/v/v/v).

Both the peaks of radioactivity (detected by scanning the thin-layer plates in a radiochromatogram scanner) and the spots of di-(*N*-formyl-D-penicillamine)disulphide (as revealed by spraying the plates with an alcoholic solution of bromocresol green) were perfectly symmetrical.

either as such, or in the presence of 0.5 nmol of heat-inactivated R61 enzyme (5 min at 100 °C), or in the presence of the same amount of active R61 enzyme, it was observed that 80–90% of the SH groups originally present remained titratable by dithio-bis-nitrobenzoic acid after incubation in the absence of enzyme or in the presence of inactivated enzyme but that 80% of these groups had disappeared after incubation in the presence of native enzyme. Thus, it is not impossible that the enzyme preparation somehow participated in the oxidation of the *N*-formyl-D-penicillamine initially released.

Comparable results were obtained with 5-¹⁴C-phenoxy-methylpenicillin which was degraded into ¹⁴C-*N*-formyl-D-penicillamine and non-radioactive phenoxyacetyl-glycine.

In summary, it seems that within the limits of experimental

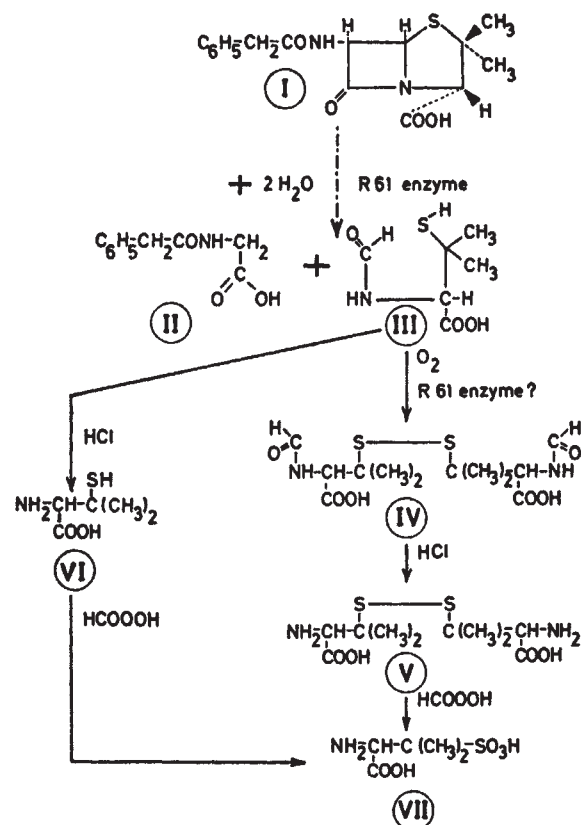


Fig. 3 Degradation of benzylpenicillin. I, benzylpenicillin; II, phenylacetyl-glycine; III, *N*-formyl-D-penicillamine; IV, di-(*N*-formyl-D-penicillamine)disulphide; V, D-penicillamine disulphide; VI, D-penicillamine; VII, dimethyl cysteic acid. Degradation of III into VI requires acid treatment (1 N HCl) during several hours at 60°C. Degradation of IV into V can be achieved by the same acid treatment during 1 h.

error, *N*-(acyl)-glycine and *N*-formyl-D-penicillamine are released in stoichiometric amounts from the complex formed between penicillin and the exocellular R61 DD-carboxypeptidase-transpeptidase, and that release of these fragments and enzyme reactivation are concomitant events. If penicillin is benzylpenicillin, phenylacetyl-glycine is released. If penicillin is phenoxymethylpenicillin, phenoxycetyl-glycine is released. Globally, the reaction consists in the addition of two H₂O molecules and results in the hydrolysis of the amide bond and in the rupture of both C5-C6 and C5-S linkages (Fig. 3). The nature of the acyl substituent on the antibiotic molecule is important; although it does not modify the pathway of the reaction, it obviously influences the half life of the complex formed between penicillin and the enzyme. The released *N*-formyl-D-penicillamine undergoes subsequent oxidation with formation of the disulphide derivative.

Studies^{7,8} with isolated membranes have shown that enzyme-penicillin complexes found in membranes do not always give rise to the products we have found with purified enzyme. Further work is required in order to unravel the exact mechanism of the reactions.

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- 1 Abraham, E. P., *Biosynthesis and Enzymic Hydrolysis of Penicillins and Cephalosporins* (University of Tokyo Press, Tokyo, 1974).
- 2 Ghuyssen, J. M., et al., *Bull. Inst. Pasteur*, 73, 101-140 (1975).
- 3 Frère, J. M., Leyh-Bouille, M., Ghuyssen, J. M., and Perkins, H. R., *Eur. J. Biochem.*, 50, 203-214 (1974).
- 4 Frère, J. M., Ghuyssen, J. M., and Iwatsubo, M., *Eur. J. Biochem.*, 57, 343-351 (1975).
- 5 Frère, J. M., Ghuyssen, J. M., Degelaen, J., Loffet, A., and Perkins, H. R., *Nature*, 258, 168-170 (1975).
- 6 Clarke, H. T., Johnson, J. R., and Robinson, R., (eds), *Chemistry of Penicillin*, 471 and 469 (Princeton University Press, Princeton, New Jersey, 1949).
- 7 Hammarström, S., and Strominger, J. L., *Proc. natn. Acad. Sci. U.S.A.*, 72, 3463-3467 (1975).
- 8 Marquet, A., Dusart, J., Ghuyssen, J. M., and Perkins, H. R., *Eur. J. Biochem.*, 46, 515-523 (1974).

Phage-coded protein prevents restriction of unmodified progeny T4 DNA

Host-controlled restriction modification systems usually prevent the survival of unmodified genomes in bacterial cells¹⁻⁴. Phage DNA can be modified by methylation (λ and $\phi 1$)⁵⁻⁷ or by glucosylation (T-even phages⁸). Modification of the λ genome is mediated by a host methylase⁸ whereas modification of the T4 genome is by phage coded α - and β -glucosyl transferases⁹. The restriction systems of *E. coli* (*rgl*) acting on non-glucosylated (glu^-) T-even phages are called r_6 and $r_{2,4}$ (ref. 10): r_6 restricts all glu^- T-even phages, whereas $r_{2,4}$ restricts only T2 and T4 glu^- phages. Hattman and Fukasawa¹¹ showed that when glu^+ T4 DNA was injected into an *rgl*⁺ uridine diphosphoglucose (UDPG) pyrophosphorylaseless mutant, the progeny glu^- DNA replicated and was matured into virions which they called T*. To explain why the replicating glu^- progeny DNA is not restricted in the *rgl*⁺ host while the entering T* virion DNA is, Fukasawa¹² postulated that the *rgl* system is localised in the membrane. Incoming glu^- parental DNA would be restricted during passage through the membrane. Incoming glu^+ parental DNA (which is not restricted) would be replicated as glu^- DNA in the absence of UDPG. Since the replicating DNA is not subject to the restriction system localised in the membrane it should not be restricted. Not surprisingly, r_6 was shown to be localised in the membrane^{13,14}. There is, however, good evidence that $r_{2,4}$ can restrict replicating DNA^{15,16} and is, at least in that sense, cytoplasmic. Thus, the question why $r_{2,4}$ does not restrict unmodified progeny DNA is still unresolved. We report here that the phage specifies a protein which prevents the action of $r_{2,4}$. The anti- $r_{2,4}$ activity specified by the modified parental genome prevents restriction of the unmodified progeny DNA by $r_{2,4}$.

Of the two restriction activities of the host, coded by the *rgl* system, r_6 seems more readily and rapidly inactivable, since it is inhibited by phage infection even in the absence of expression of the phage genome^{13,14}. Table 1 shows that the inactivation of $r_{2,4}$, in contrast to r_6 , requires expression of the phage genome. Infection of $r_6^+r_{2,4}^-$ cells inactivates the r_6 activity in the presence or absence of primary phage expression. In $r_{2,4}^+$

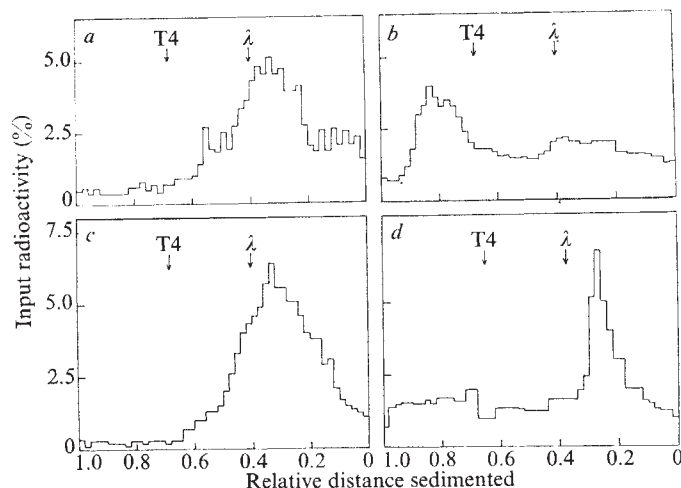


Fig. 1 Demonstration of a protein inhibiting the cleavage of superinfecting *glu*⁻ T4 DNA. *rgl*⁺ *exo V*⁻ (W3110 *recB*⁻) cells infected with T4 *imm*⁻ *s*⁻ phage (which permit less leakage of *imm* expression late in infection¹⁸) in the presence or absence of chloramphenicol, were superinfected with ³²P-*glu*⁻ T4 (T4α *gt*^{am8} *βgt*^{am10} *K rgl*⁻ *su*⁻) after 10 min. Five minutes after superinfection, native DNA molecules were extracted²⁰ and analysed in neutral sucrose gradients. The same preparations, denatured by adding sodium hydroxide to 0.4 N, were also analysed in alkaline sucrose gradients¹⁶. Centrifugation was for 110 min at 147,000g at 20 °C. ³H-λC₁₈₅₇SusS₇ particles¹⁹ were added to the infected *E. coli* during lysis of spheroplasts by detergent, to serve as a marker. The calculated²¹ position of T4 DNA was confirmed on several occasions. *a*, Chloramphenicol absent, alkaline gradient; *b*, chloramphenicol absent, neutral gradient; *c*, chloramphenicol present, alkaline gradient; *d*, chloramphenicol present, neutral gradient.

cells the primary infecting *imm*⁻ phage prevents the breakdown of superinfecting *glu*⁻ phage, only when primary phage expression takes place. In these cells, when primary phage expression is prevented by chloramphenicol or ultraviolet irradiation (UV), of the primary infecting virions the superinfecting *glu*⁻ T4 is broken down. This implies that the primary infecting phage codes for a protein which prevents the breakdown of *glu*⁻ T4 DNA. We have shown that the restriction of unmodified T4 *in vivo* takes place in two steps¹⁶. The first step caused by the restriction endonuclease, which we call cleavage, results in double-stranded breaks in the unmodified genome. The large trichloroacetic acid (TCA)-insoluble double-stranded pieces are further degraded by exonuclease (*exo*) V (the *rec BC* enzyme) to small acid-soluble and insoluble fragments^{13,16}. We call this second step DNA breakdown.

T4 makes a protein which prevents *exo V* action^{17,18}. In the experiment described above we measured only DNA breakdown. The inhibition of breakdown by the primary phage-coded proteins may reflect either inhibition of *exo V* or inhibition of cleavage by restriction enzymes or both. To see whether cleavage was inhibited, we measured the cleavage reaction alone in *exo V*⁻ hosts. *E. coli rgl*⁺ infected with T4 *imm*⁻ *s*⁻ phage, permits superinfection by *glu*⁻ T4 phage without DNA breakdown¹³. Figure 1 shows the fate of this superinfecting phage DNA. When sedimented on an alkaline sucrose gradient the strands are shown to be fragmented to 1/4–1/20 their original length, as might be expected for restriction cleavage of *glu*⁻ DNA by *rgl* (Fig. 1*a*). But this same DNA, when native, sediments mainly as concatemers (Fig. 1*b*). This, however, is the result expected for *glu*⁻ DNA on a phenotypically *rgl*⁻ host, that is if *r*_{2,4} were inactivated. When this

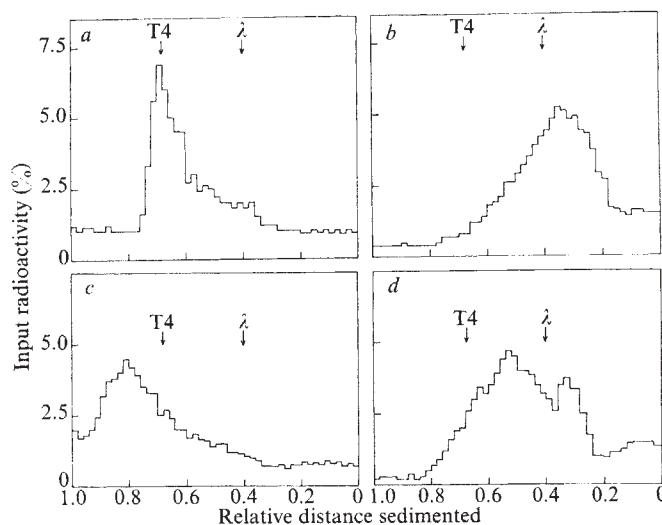


Fig. 2 Demonstration of a chloramphenicol-sensitive nicking enzyme in T4 *imm*⁻ *s*⁻ infected cells. Ten minutes after primary infection with T4 *imm*⁻ *s*⁻ phage the cells were superinfected with the appropriate ³²P-phage. Five minutes after superinfection DNA was extracted and analysed for size distribution in native and denatured conditions. Experimental details are described in legend for Fig. 1. *a*, *E. coli K rgl*⁻ cells superinfected with *glu*⁻T4, chloramphenicol present, alkaline gradient; *b*, *E. coli K rgl*⁻ cells superinfected with *glu*⁻T4, chloramphenicol absent, alkaline gradient; *c*, *E. coli K rgl*⁻ cells superinfected with *glu*⁻T4, chloramphenicol absent, neutral gradient; *d*, *E. coli K rgl*⁺ *exo V*⁻ cells, superinfected with T4 wild-type phage, chloramphenicol absent, alkaline gradient.

Table 1 Effect of primary infecting phage-coded function(s) in the breakdown of superinfecting *glu*⁻ T4

Bacterial strains	Chloramphenicol	Primary phage	Superinfecting phage	% DNA breakdown
<i>r</i> ₆ ⁻ <i>r</i> _{2,4} ⁻	—	T4 <i>glu</i> ⁻	—	4
	—	T4 <i>imm</i> ⁻	T4 <i>glu</i> ⁻	3
	+	T4 <i>imm</i> ⁻	T4 <i>glu</i> ⁻	3
	—	UV.T4	T4 <i>glu</i> ⁻	14
<i>r</i> ₆ ⁺ <i>r</i> _{2,4} ⁻	—	T4 <i>glu</i> ⁻	—	28
	—	T4 <i>imm</i> ⁻	T4 <i>glu</i> ⁻	4
	+	T4 <i>imm</i> ⁻	T4 <i>glu</i> ⁻	3
	—	UV.T4	T4 <i>glu</i> ⁻	16
<i>r</i> ₆ ⁻ <i>r</i> _{2,4} ⁺	—	T4 <i>glu</i> ⁻	—	30
	—	T4 <i>imm</i> ⁻	T4 <i>glu</i> ⁻	7
	+	T4 <i>imm</i> ⁻	T4 <i>glu</i> ⁻	28
	—	UV.T4	T4 <i>glu</i> ⁻	43
<i>r</i> ₆ ⁺ <i>r</i> _{2,4} ⁺	—	T4 <i>glu</i> ⁻	—	35
	—	T4 <i>imm</i> ⁻	T4 <i>glu</i> ⁻	5
	+	T4 <i>imm</i> ⁻	T4 <i>glu</i> ⁻	36
	—	UV.T4	T4 <i>glu</i> ⁻	35

Phage and bacterial strains, culture conditions and other techniques used in this study are described previously¹⁶. Log phase cells (5×10^8 cells per ml) were infected with 3–4 T4 *imm*⁻ phage per bacterium. 2–3 ³²P-*glu*⁻ T4 was added 5 min after primary infection. DNA breakdown was determined as trichloroacetic acid (TCA)-soluble fraction of DNA, 5 min after superinfection. Chloramphenicol (100 μg ml⁻¹), when required was added 3 min before primary infection. The data given are not corrected for adsorption, which was 85–95%.

experiment is repeated in the presence of chloramphenicol not only are nicks observed in the isolated strands (Fig. 1c) but double-stranded cleavage (Fig. 1d) is observed, as expected for glu^- DNA in an rgl^+ host. Thus it seems that the cleavage activity of $r_{2,4}$ on double-stranded DNA is inhibited by a primary infecting phage-coded protein, the synthesis of which is inhibited by chloramphenicol.

To be certain that nicking in Fig. 1c was caused by $r_{2,4}$, we repeated the experiment using an $\text{rgl}^-(r_6^-r_{2,4}^-)$ host cell. rgl^- cells infected with T4 imm^-s^- in the presence of chloramphenicol were superinfected with glu^- T4. Single strands of DNA sediment mainly as whole strands (Fig. 2a), but in the absence of chloramphenicol the superinfecting glu^- DNA is nicked (Fig. 2b). Thus there are two different nicking (endonuclease) activities in the cell at different times. The first, the host rgl system, nicks complementary DNA strands in close proximity leading to DNA cleavage. This cleavage activity of the host rgl system can be demonstrated even in the presence of chloramphenicol in rgl^+ cells but not in rgl^- cells in the absence or presence of chloramphenicol (compare Figs 1d and 2c). The host cleavage activity is inhibited by a phage-coded protein which appears after primary infection and is not made in the presence of chloramphenicol (compare Fig. 1b and d). The second, a phage-coded enzyme, produces single-stranded nicks but does not cleave double-stranded DNA. We think that this enzyme is probably the phage endonuclease demonstrated *in vitro* by Altman and Meselson²². Thus, in the absence of chloramphenicol, when rgl cleavage activity is inhibited, nicking activity is still apparent (compare Fig. 1a and c). This activity is independent of the rgl system and requires phage protein synthesis (compare Fig. 2a and b). The nicking activity is also not greatly affected by glucosylation since both glu^+ and glu^- DNA are nicked in these conditions

(compare Figs 2d and 1a). In any case, data in Figs 1 and 2 show that a protein is made, after primary phage infection, which prevents initial cleavage of superinfecting glu^- DNA by $r_{2,4}$. We call this protein anti- $r_{2,4}$.

To establish the time of appearance of the anti- $r_{2,4}$ activity we infected *E. coli* rgl^+ with T4 imm^-s^- phage and superinfected with glu^- T4 at various times after primary infection. Figure 3 shows that large molecules, of mature T4 DNA size or larger, accumulate as the time of superinfection is delayed. This anti- $r_{2,4}$ activity appears quite soon after infection. The exact time of its appearance is difficult to establish in these experiments, however, since it is still being synthesised between the time of superinfection and completion of cleavage of the superinfecting glu^- phage DNA. Therefore, the 1-min point has probably allowed more than 1 min of expression by the primary phage. By 7 min after infection, when unmodified progeny DNA synthesis is expected to start, $r_{2,4}$ activity has disappeared.

An intriguing problem in the restriction system of glu^- T4 is the successful replication of intracellular unmodified genomes in contrast to the complete restriction of entering parental unmodified genomes. The demonstration of the anti- $r_{2,4}$ activity gives a satisfactory explanation for the resistance of unmodified progeny genomes to restriction. This does not seem to be so in the case of phage λ . The analogous experiment, infection of restricting bacteria (r_K^+) by modified λ in the presence of an inhibitor of S-adenosylmethionine (SAM) production, leads to the production of normal unmodified phage²³. SAM (unlike UDPG in the case of T4) is, however, needed for restriction as well as modification⁸. When a similar experiment was repeated using a P1 lysogen, the λ progeny were restricted as SAM is needed for P1 modification but not for restriction²³. This experiment also shows that no inhibitor of the P1 restriction enzyme is made in this case. Nevertheless it is possible that complementation of modified λ by superinfecting unmodified λ in a non-permissive host²⁴ may involve production of anti- r_K . Direct measurement *in vivo*, of cleavage activity by r_K (ref. 16) in these conditions should decide this question. It is probable, however, that complementation by cleaved unmodified fragments is enhanced by the inactivation of exo V^{25} by the gamma gene-product from the primary infecting modified λ phage²⁶.

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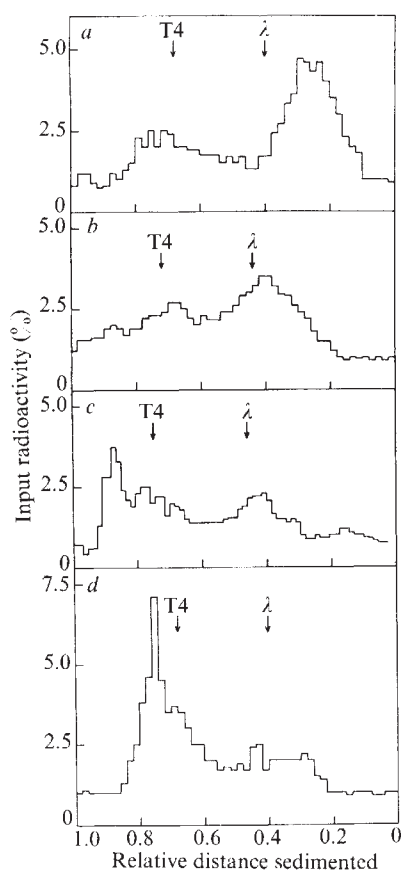


Fig. 3 Time of appearance of anti- $r_{2,4}$. rgl^+ *exo V* cells infected with T4 imm^-s^- phage were superinfected with ^{32}P - glu^- T4 at various times. Native DNA was analysed 5 min after superinfection. a, b, c and d were superinfected at 1, 2, 5 and 7 min after primary infection respectively.

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- Luria, S. E., and Human, H. L., *J. Bact.*, **64**, 557-569 (1952).
- Bertani, G., and Wiegler, J. J., *J. Bact.*, **65**, 113-121 (1953).
- Revel, H. R., and Luria, S. E., *A. Rev. Genet.*, **4**, 177-192 (1970).
- Arber, W., in *Progress in Nucleic Acid Research and Molecular Biology*, **14** (edit. by Cohn, W. E.), 1-34 (Academic, New York, 1974).
- Arber, W., *J. molec. Biol.*, **11**, 247-256 (1965).
- Kuhnlein, K., and Arber, W., *J. molec. Biol.*, **63**, 9-19 (1972).
- Nathans, D., and Smith, H. O., *A. Rev. Biochem.*, **44**, 273-293 (1975).
- Meselson, M., Yuan, R., and Heywood, J., *A. Rev. Biochem.*, **44**, 447-466 (1972).
- Kornberg, S. R., Zimmerman, S. B., and Kornberg, A., *J. biol. Chem.*, **236**, 1487-1493 (1961).
- Revel, H. R., *Virology*, **31**, 688-701 (1967).
- Hattman, S., and Fukasawa, T., *Proc. natn. Acad. Sci. U.S.A.*, **50**, 297-305 (1963).
- Fukasawa, T., *J. molec. Biol.*, **9**, 525-536 (1964).
- Silverstein, J., and Goldberg, E. B., *Virology* (in the press).
- Hewlett, J., and Mathews, C. K., *J. Virol.*, **15**, 776-784 (1975).
- Fleischman, R. A., and Richardson, C. C., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2527-2531 (1971).
- Dharmalingam, K., and Goldberg, E. B., *Nature*, **260**, 000-000 (1976).
- Tanner, D., and Oishi, M., *Biochim. biophys. Acta*, **228**, 764-769 (1971).
- Sakaki, Y., *J. Virol.*, **15**, 1611-1612 (1974).
- Cornett, J. B., *J. Virol.*, **13**, 312-321 (1974).
- Shah, D. H., and Berger, H., *J. molec. Biol.*, **57**, 17-34 (1971).
- Studier, F. W., *J. molec. Biol.*, **11**, 373-390 (1965).
- Altman, S., and Meselson, M., *Proc. natn. Acad. Sci. U.S.A.*, **66**, 716-721 (1970).
- Lark, C., and Arber, W., *J. molec. Biol.*, **52**, 337-348 (1970).
- Heib, J., Rolfe, B., and Shell, J., *Virology*, **59**, 356-370 (1974).
- Silverstein, J., and Goldberg, E. B., *Virology* (in the press).
- Zissler, J., Signer, E. R., and Schaefer, F., in *The Bacteriophage Lambda* (edit by Hershey, A. D.), 455-468 (Cold Spring Harbor Laboratory, New York, 1971).

matters arising

Tornado forum

RECENTLY the proposal was made that motor traffic has contributed significantly to the sixfold increase in incidence of tornadoes in the USA in the past forty years.¹ The authors saw the vorticity introduced into the atmosphere by traffic driving on the right as favouring the generation of cyclonic tornadoes, and they were encouraged in this hypothesis by an increase in the proportion of cyclonic to anticyclonic tornadoes, and also by the low incidence of tornadoes reported on Saturdays, when traffic would be lower and more unidirectional (away from large cities).

The suggestion has provoked many responses and to publish them all would have occupied more space than we could allot. So we print below abridged versions of some of the communications received, followed by a reply from the authors.

¹ Isaacs, J. D., Stork, J. W., Goldstein, D. B., and Wick, G. L., *Nature*, 253, 254-255 (1975).

A MOTOR vehicle or any other projectile in air produces vortices in its wake, but no net vorticity. If there are anticyclonic vortices along edges of the highway, as shown in Fig. 1 of Isaacs *et al.*, their vorticity must be balanced by cyclonic vorticity in the median strip.

The width of a highway is small compared to a cloud, and a significant updraft entraining the vortices produced by motor vehicles would realise no net contribution from that source. The main atmospheric phenomena produced by cars are turbulence, and gaseous and particulate combustion products along the highway, dissipating and diffusing downwind.

The low incidence of tornadoes on Saturdays perhaps reflects some unfortunate vagaries in reporting. Or, conceivably, changes in the atmospheric radiation budget and aerosol content induced by weekend factory closings and lowered air pollution produce changes in static stability and cloud particle development.

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WE believe that the mechanism for vorticity generation and accumulation in the atmosphere proposed by Isaacs *et al.* is based on misconceptions about the nature

of vorticity. The atmosphere contains background vorticity arising from the rotation of the Earth and relative vorticity from weather disturbances and boundary effects, including moving vehicles. In fact the background vorticity can be ignored to high accuracy in the dynamics of flow around vehicles, since Coriolis effects are minute. The following argument depends, however, mainly on kinematical results, which are exact.

We begin by considering the generation of vorticity by a single moving vehicle. Absolute vorticity can be generated in a homogeneous fluid only at boundary surfaces moving relative to the fluid¹, and so is generated at the sides, top and bottom of the vehicle, at its wheels and at the ground in its neighbourhood. Once generated it is advected with the air stream into the vehicle's wake. The vortex filaments of the resulting relative vorticity vector field ω must either form closed loops or terminate at a boundary rotating relative to the Earth; and on this basis we can distinguish two constituents in the relative vorticity generated at a moving vehicle:

- (1) When the vehicle moves in a straight line, closed vortex loops are shed continuously into its wake. These profoundly affect local levels of vorticity close behind the vehicle, but have no effect whatever either on the circulation round a contour enclosing the wake, or on the bulk integral

$$\int_V \omega dV$$

over a volume V including the wake and the neighbourhood of the vehicle, because each vortex filament is closed. (None of these facts is changed by the presence within V of more vehicles whether driving on the right or on the left.)

- (2) Vorticity having a net vertical component is generated when a vehicle turns, at a rate proportional to minus the rate of change of angular velocity of the vehicle about the vertical; see equation (1). There is an additional net horizontal component associated with the rotation of the wheels, which need not concern us further.

It is immediately clear that randomly varying vehicle movements can have no systematic gross effect over a large area as claimed by Isaacs *et al.* The vehicles could, however, produce localised concentrations of vorticity (changing sign

over length scales of a few metres) the significance of which will depend very much on persistence and hence on inter-diffusion rates. We shall discuss this question further elsewhere, but remark here that order-of-magnitude estimates based on plausible levels of turbulence suggest persistence times of minutes rather than hours, and vortices of dust devil rather than tornado scale.

If, as an opposite extreme, a large number of vehicles were to be driven simultaneously in fast clockwise circles, there would indeed temporarily be a cyclonic (that is, anticlockwise) contribution to the bulk vorticity integral. But this can be shown to be associated entirely with the air below vehicle rooftop level or, more precisely, below a plane surface s lying just above all the vehicles. When summed over the vast bulk of the atmosphere lying above s , the total component of vorticity normal to s caused by the vehicles is, and must remain, exactly zero (see equation (2) below). This is a consequence of the simple fact that each vehicle-generated vortex filament passing upward through s must go back down through s at another point, possibly terminating on a moving surface such as the underside of the same vehicle.

In summary, neither mechanism (1) nor (2) can plausibly be connected with the generation of tornadoes, which takes place high above the ground and which involves air motions coherent over length scales of kilometres.

Isaacs *et al.* stated that cyclonic vorticity "is generated by the torque between the two opposing streams of traffic", and this led to their view that it is better to drive on the left than the right of the road. Unfortunately torque does not determine the generation of vorticity, either locally or globally. Although doubling the separation of two traffic streams may approximately double the torque (to say nothing of the far larger contribution from unidirectional traffic on widely-separated highways) it has absolutely no effect on the net generation of vorticity. The vorticity due to the traffic streams is, to an excellent approximation, the sum of the contributions produced at the surface of each vehicle, locally redistributed by advection and diffusion; and any effects on the bulk vorticity integral have nothing to do with the separation of traffic streams, nor with left as against right hand drive.

The basic mathematical fact underlying our discussion is the solenoidal property of ω : since ω is the curl of a velocity

field, $\nabla \cdot \omega$ is identically zero. It follows that

$$\begin{aligned}\int_V \omega_i dV &= \int_V \frac{\partial}{\partial x_j} (x_i \omega_j) dV \\ &= \int_S x_i (\omega \cdot \mathbf{n}) dS\end{aligned}$$

where V is a suitable piece of the atmosphere, partially bounded by the surface S , comprising the ground and the surfaces of stationary and moving objects (vehicles, their wheels, aeroplanes) which are envisaged as the source of the contribution ω to the relative vorticity, itself supposed confined within V . From this identity it may readily be shown that

$$\int_V \omega dV = -2 \sum \Omega_r(t) V_r \quad (1)$$

where $\Omega_r(t)$ is the angular velocity of the r th moving body, and V_r its volume. We here use the fact, fundamental to the vorticity generation mechanism, that the no slip condition¹ applies at all parts of the surface S .

Further, if all the moving objects lie below a plane surface s then, if ω_s is the component of ω normal to s and v is the part of V above s ,

$$\int_s \omega_s dS = - \int_v \nabla \cdot \omega dV = 0$$

Hence, by integration with respect to x_3 ,

$$\int_v \omega_3 dV = 0 \quad (2)$$

The results (1) and (2) are both exact, even for an atmosphere in which the motion is baroclinic, compressible, and turbulent.

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¹ Batchelor, G. K., *An Introduction to Fluid Dynamics* 149, 277 (Cambridge University Press, 1967).

ISAACS *et al.* assert that the opposing streams of traffic on a road induce a net cyclonic vorticity in the atmosphere. Because vorticity diffuses exponentially slowly at large distances from a source, however, this is not so.

If the opposing traffic streams are modelled by infinite plates a distance D apart, then the resulting mean flow field at any finite time after the motion begins is of the form shown in Fig. 1. The

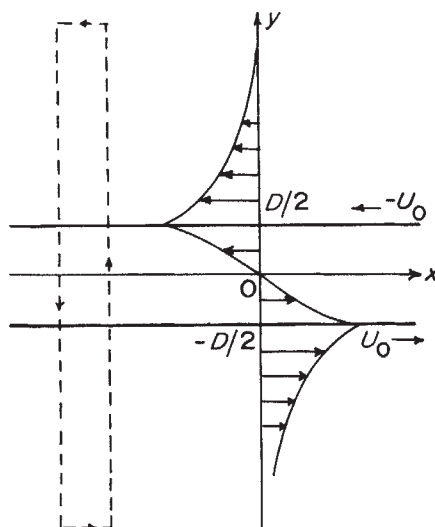


Fig. 1 Mean velocity $U(y)$ associated with the motion of two infinite plates ($y = D/2$ and $-D/2$) travelling at speed U_0 in opposite directions.

detailed behaviour of the mean velocity profile near the plates is unimportant. Although the motion near the plates is turbulent in general, any far-field disturbance must propagate normal to the plates by the action of molecular diffusion, and so the mean velocity decays exponentially as $y \rightarrow \infty$. Thus, integrating the mean vorticity $\partial U / \partial y$ with respect to y , we find that the total vorticity per unit distance along the plates in the region $|y| > D/2$ is finite and equal to $2U_0$, whereas the total vorticity in $|y| < D/2$ is equal to $-2U_0$, where U_0 is the speed of each plate. Thus the cyclonic vorticity in the region between the plates is just balanced by the anticyclonic vorticity outside the plates. Alternatively, we see that the net circulation around a closed contour (as shown by the dotted line in Fig. 1) goes to zero as the y dimension of the contour increases. This is because the cyclonic torque induced by the plates is opposed by the inertia of the fluid at infinity, which is at rest.

This result implies that although cyclonic vorticity is generated between the streams of traffic, there is no net generation of vorticity, because an equal amount of anticyclonic vorticity is also induced. It would seem therefore that, according to the hypothesis of Isaacs *et al.*, either both cyclonic and anticyclonic tornadoes ought to be equally probable, or no tornadoes ought to be generated, depending on whether the air is not, or is mixed horizontally in the process of tornado generation.

On the other hand, Isaacs *et al.* do give some strong circumstantial evidence for the connection between cyclonic tornadoes and traffic-induced vorticity. Their hypothesis can perhaps still be retained if the

second ingredient involved in the generation of tornadoes, namely convection, is included. Any vorticity induced by traffic is initially confined to a layer a couple of metres deep near the ground. To produce a tornado, this vorticity must be preferentially advected vertically. Such motions are found in convection, which is an anisotropic process where horizontal mixing is suppressed¹. Moreover, road pavement in general absorbs more solar radiation than does the surrounding terrain and so the road is expected to be a stronger source of convection than the surrounding terrain. (The cars themselves also act as a local heat source.) Convection therefore ought to occur preferentially from the road. Because the cyclonic vorticity (of mean strength $2U_0/D$) is concentrated over the road while the anticyclonic vorticity is diffused on each side of the road, it would appear that the convection leads to the preferential advection of cyclonic vorticity into the troposphere, as required by Isaacs *et al.* That is, cyclonic tornadoes can be produced because roads are sources of both cyclonic vorticity and convection.

This work was done in the Department of Mathematics, University of British Columbia, and was supported in part by the NRC.

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¹ Manton, M. J., *Aust. J. Phys.*, 27, 495 (1974).

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A CALCULATION is indicated, though not shown, by Isaacs *et al.*, purporting to prove that motor vehicle traffic contributes significantly to the atmospheric vorticity over the USA and thereby leads to a more homogeneously cyclonic troposphere and consequently to changes in the broad scale spatial and temporal distribution and frequency of tornadoes. In the following I have attempted to repeat the calculation, with results substantially different from those of the authors.

A typical round trip is taken to be a straight line of length L , a straight line return and a U-turn at each end, with a separation between lanes of width d . If the average force exerted backwards by the wheels on the road is F , then the net anticyclonic angular momentum added to the earth, located at the centroid of the path, is $2FLd/V$, where V is the automobile speed, with nominally equal contributions coming from the straight legs and the U-turns. We assume the force F to be the air drag of the vehicle, given by

$$F = \rho a C_D V^2 / 2 \quad (1)$$

where ρ is the air density, a is the cross-sectional area of the vehicle, and C_D is the drag coefficient, taken to be 0.5.

Since the speed while making the U-turns is likely to be much less than that on the straight legs, we ignore those contributions, and the average torque produced by the vehicle is then

$$T = Fd = \rho a C_D V^2 d/2 \quad (2)$$

Isaacs *et al.* assume that 2×10^6 automobiles are on the road in the USA at any time, and that the contributions to torque from autos and trucks are equal. Using these figures, and assuming a mean cross-sectional area of 3 m^2 , $V = 20 \text{ ms}^{-1}$, the lane spacing $d = 20 \text{ m}$, and $\rho = 1.3 \text{ kg m}^{-3}$, we obtain a net torque production of $3.2 \times 10^{10} \text{ kg m}^2 \text{ s}^{-2}$. In this calculation, the difference in direction between the torques produced in different parts of the USA is ignored, which amounts to assuming the cosine of angles smaller than $\sim 20^\circ$ to be unity.

The angular momentum, M , of a rotating body is the second radial moment of its vorticity

$$M = \int \zeta r^2 dm$$

where ζ is the vertical component of vorticity and dm is the mass differential. Over the USA during most seasons, the mean vorticity of the atmosphere is close to the vertical component of the Earth's vorticity, $f \approx 10^{-4} \text{ s}^{-1}$. Thus the angular momentum of the atmosphere of the USA is roughly

$$M = (p_0/g) A f r^2/2,$$

where p_0 is the mean surface pressure, g is the acceleration of gravity, A is the area of the USA, and r is the mean radius, corresponding to the moment of inertia of the USA, which we estimate to be 2,000 km. Taking p_0/g to be 10^4 kg m^{-2} and $A = 8 \times 10^{12} \text{ m}^2$, we obtain $M = 1.6 \times 10^{25} \text{ kg m}^2 \text{ s}^{-1}$. Thus it would seem that the motor vehicle torque would have to continue for 10^{14} s , about 3 Myr, to show a noticeable effect. The natural turnover time of angular momentum in the atmosphere is of order days or weeks, however, so that the effects suggested by Isaacs *et al.* seem very unlikely.

About the largest effect that could conceivably be produced in the way the authors envisage would occur if all the vehicles in the USA drove in the same direction along highways close to the USA coastlines and borders. In this case the effect would be increased by a factor of 10^5 , but would still be too small to be noticeable.

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THERE is a Saturday anomaly in the reporting procedures that affects the final numbers in the paper of Isaacs *et al.*

The major source of information about tornadoes in the USA since 1953–54 has been press clippings from out-state newspapers. This drastic change in data-gathering techniques can best be seen in the figure on p. 35 of ref. 1. The average annual totals jumped from $\sim 280 \text{ yr}^{-1}$ to 640 yr^{-1} beginning in 1954. In Missouri the use of press clipping information coincided with a change in the average annual total reports from 13 yr^{-1} to 33 yr^{-1} . One might conclude, therefore, that in Missouri almost two-thirds of the total reports are strongly dependent on press clipping information. For the entire USA it would seem that more than half of the total number of reports depend to some extent on press clippings.

In general, these additional tornado reports generated by press clipping information tend to be the smaller, short lived mini-tornadoes in largely rural areas. The small amount of property damage and infrequent personal injuries does not make them newsworthy items for the large metropolitan dailies. These storm reports are frequently covered only by local weeklies and the smaller out-state 'daily' papers. It is in these 'dailies' that the anomaly on Saturdays has its origins.

A substantial percentage of out-state daily papers do not publish on Sundays, and a surprising number do not publish on both Saturday and Sunday. In Missouri, for example, 45 of the 61 daily papers do not publish on Sunday and 24 of 61 do not publish on both Saturday and Sunday. Thus, the events of Saturday are frequently old news by the time these dailies go to press on Monday. The minor tornado activity that occurs on Saturday is not nearly as intensively covered as a similar occurrence on any other day of the week. This discontinuity in the basic source of information for tornado reports could easily account for a decrease in Saturday totals of ~ 10 –15%. When up to two-thirds of the basic information sources for more than half of the usual total reports cut back to 25–50% efficiency for Saturday events, we end up with an anomaly.

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¹ Court, A., *Tornado Incidence Maps, ESSA Tech. Memo. ERLTM-NSSL, 49* (1970).

THE evidence given by Isaacs *et al.* for their discovery is that, first, "the annual centre of tornado intensity weighted by area . . . has increasingly occupied the eastern portion of the continent", and second that of those few tornadoes for which rotation sense was reported, during the past 12 yr (1962–1973) 8.1% were anticyclonic compared with only 36.4% in the preceding 12 yr; and third,

especially, that only 1,868 of 15,234 tornadoes during 22 yr occurred on Saturdays.

Tornadoes are reported by people according to damage done, and as the US population expanded westwards so did tornado reports. Since 1925, the apparent centre of tornado incidence has moved generally south-westwards into eastern Kansas (by 1925), to central Kansas (by 1951), and to central Oklahoma (by 1963). Oklahoma, now the region of greatest incidence, was shown as tornado deficient on earlier maps because it was the last Plains state to be settled¹.

Rotation sense is given for too few tornadoes to be of significance; the big surprise is that any anticyclonic tornadoes whatever are reported. "Motor vehicles are the only man-made source of non-random vorticity we know", the authors say. Perhaps they have never seen two railway trains pass at full speed, or noted the action of exhaust fans and rooftop ventilators.

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ISAACS*, STORK AND WICK* REPLY—Opposing streams of traffic in the USA exert anticyclonic torques on the solid Earth and, from action and reaction, compensating cyclonic torques on the atmosphere. These torques tend to slow down the Earth's rotation and to increase the cyclonic rotation of the local atmosphere in conservation of angular momentum.

We agree with Kessler¹, Morton *et al.*² and Manton³ that there is zero net vorticity in the total atmosphere caused by traffic. Our original discussion was based on vorticity rather than angular momentum. We were in error in not recognising that a discussion of the process from a consideration of vorticity is much less direct than from a consideration of angular momentum. Angular momentum is a more fundamental quantity in the formation of strongly rotating storms.

There can be little doubt that US automotive traffic introduces cyclonic angular momentum into the local atmosphere, which can participate in the formation of tornadoes. To illustrate how this occurs, consider this example: if you set fluid in a bowl into circulation by periodically immersing your hands and moving them past one another in straight lines always in the same relative direction but in any orientation and location, the fluid will gain angular momentum. Yet for this same fluid the no-slip condition at the boundaries requires zero net vorticity. The boundary

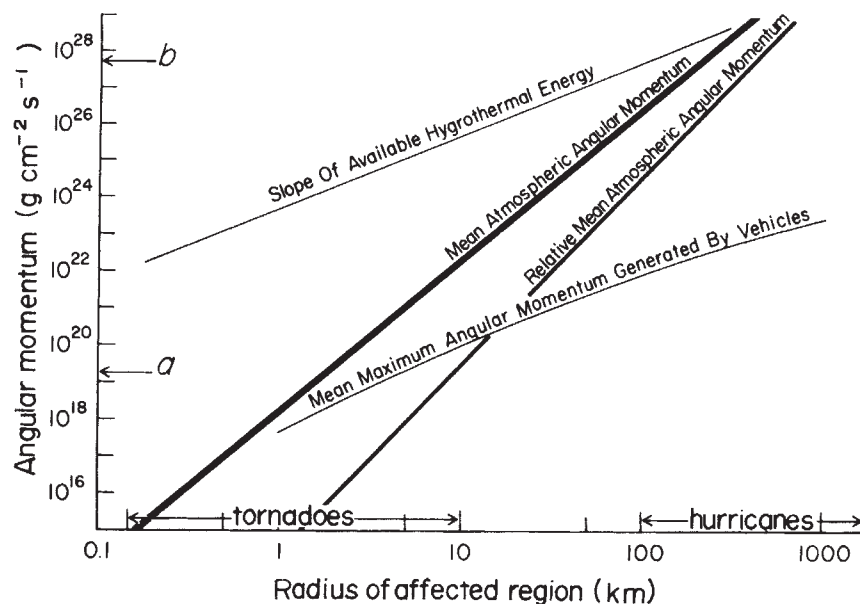


Fig. 1 Atmospheric angular momentum as a function of the affected area and anthropogenic input. The mean atmospheric angular momentum contained in a mass of air is that due to its planetary rotation around its central axis and is involved in radial convergence. It scales as the fourth power of the radius of the affected area. The mean relative atmospheric angular momentum is that involved in linear convergence over the dimensions on the abscissa, and scales as the fifth power of the radius. (Both are at $\sim 45^\circ$ latitude.) The angular momentum generated by the vehicles is based on the area, the residence time of air over the region (10^5 – 10^6 s) and the maximum density of traffic within such an area. The dimensions involved in the convection of a tornado or hurricane are indicated on the abscissa. The points labelled *a* and *b* indicate the angular momentum of a single giant tornado and of a hurricane, respectively. For a similar atmosphere and similar equilibrium altitude, the hygrothermal energy available to convective storms scales as the square of the radius of the affected region. (The line is drawn with an arbitrary ordinate intercept.)

vorticity does not preclude the formation of a vortex when the fluid is allowed to flow through an orifice in the bottom of the bowl. The important points are that angular momentum can be introduced by linear motions in this way, and that it can participate in a vortex while the net vorticity remains zero.

The apparent paradox of automobile-generated angular momentum with zero vorticity is probably resolved through several mechanisms in the atmosphere. As in the example of the bowl, the boundary vorticity in the atmosphere may propagate and act outside the area of convergence, as we originally proposed. The resolution may not be unrelated to our recent observation alongside a highway bearing heavy traffic that anticyclonic filaments of small-scale rotation are embedded in large-scale cyclonic rotation.

At any rate, our calculations of the torque and resulting angular momentum agree with those of Lilly⁴, but are more conservative by a factor of about eight. We do not consider, as he did, that the torque on the atmosphere be applied to the entire air mass over the USA as though the atmosphere were a solid body. Rather this torque must exert its influence locally, and in areas consonant with those involved in the convective processes of a tornadic storm. Furthermore, if variations in local vehicle concentrations of two orders of magnitude are taken into

account, our calculations show that vehicle-produced torque, over reasonable periods of time, can produce angular momentum of the same order or greater than the natural background (see Fig. 1). The angular momentum introduced annually by vehicles is sufficient to account for that present in $\sim 10^5$ giant tornadoes. (A total of ~ 600 including all sizes are actually reported per year.) Court's point⁵ that the centre of tornado occurrence has moved westward makes it even more impressive that the centre of tornado intensity, which is what we reported, has increasingly occupied the eastern sections of the USA. The obvious inference here is that the more intense tornadoes have been increasingly found in the east in support of our original thesis.

We agree that too few observations of the rotational sense of tornadoes have been reported to make these data highly significant. A χ^2 test based on these data shows, however, a statistically significant shift from anticyclonic to cyclonic tornadoes over the 24-yr period, and thus these data support our hypothesis. The fact that anticyclonic tornadoes exist at all is important in showing that local effects other than planetary rotation can be significant for the formation of tornadoes. One of these local effects could be vehicle-generated cyclonic angular momentum.

Darkow⁶ raises an interesting point in attributing the weekly periodicity of the tornado data to periodicity in newspaper reporting. The possibility of reporting peculiarities being the cause has also concerned us. Before our initial publication, one of us contacted the Administrator of the National Oceanic and Atmospheric Administration, who was certain that weekend reporting was adequate or even intensified because of increased exposure to more people at weekends. In addition, since the data on the tornado tape we acquired from the National Severe Storms Forecast Center (NSSFC) are listed by source, we were able to separate out those that were identified as press clipping data (33%). The remaining data showed an even stronger weekly periodicity than reported for the total. These remaining data are considered to be the most reliable data by NSSFC.

As a further check we had plotted tornado injuries, damage, tornado days, path area and sums of *f*-scale numbers by day of week. We thought that tornadoes of sufficient significance to require the reporting of these parameters would not be subject to such reporting vagaries as might affect the reporting of lesser tornadoes. In every case Saturday was low, thus increasing our confidence in the validity of the findings.

Weekend changes in the production of factory wastes could conceivably influence the static stability and cloud-particle development as suggested by Kessler¹. It is difficult, however, to see why this phenomenon would have its effect only on Saturday and not on Sunday. Presumably a factory that is closed on Saturday will remain so on most of Sunday. As a further check, we have obtained a tape of thunderstorm data from NSSFC and analysed it for weekly periodicity. We found none. Thus it seems that the periodicity in tornado occurrence is not dependent on convective storm periodicity but more probably on the periodicity of input of that unique and essential ingredient of tornadoes—angular momentum.

Referring again to Fig. 1, the planetary angular momentum scales by the fourth or fifth power of the radius of the area involved in convection, depending on the type of convergence, while energy scales only as the second power of the radius. Thus a strong argument can be made that, if energy limits the population of strongly rotating convective storms of the magnitude of hurricanes (which seems to be the case), storms of the magnitude of tornadoes are comparatively deficient in planetary angular momentum, as related to energy by at least three orders of magnitude. The fact that most thunderstorms do not involve tornadoes seems to support this statement. This argues that angular momentum, rather than energy, limits the population of tornadoes, and that any input of cyclonic angular

momentum increases their incidence, as we have proposed.

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- 1 Kessler, E., *Nature*, 260, 457 (1976).
- 2 Morton, B. R., Smith, R. K., and McIntyre, M. E., *Nature*, 260, 457-458 (1976).
- 3 Manton, M. J., *Nature*, 260, 458 (1976).
- 4 Lilly, D. K., *Nature*, 260, 458-459 (1976).
- 5 Court, A., *Nature*, 260, 459 (1976).
- 6 Darkow, G. L., *Nature*, 260, 459 (1976).

Identification of averaged auditory evoked potentials in man

SAYERS *et al.*^{1,2}, in their work on the Fourier analysis of averaged auditory evoked potentials (AEP) have suggested that suppositions for arithmetic averaging are violated, since there are no differences between the mean powers in the pre- and post-stimulus averaged potentials. Their results contradict the fact that the amplitudes of AEP seem much more pronounced in comparison with averaged spontaneous EEG activity.

Fourier analyses of averaged potentials (Fig. 1) show that the forms of both amplitude and phase spectra in the post-stimulus period, where the AEP can be seen, differ significantly from those in the pre-stimulus period, where no response occurs. The maximum in the amplitude spectra of post-stimulus averaged signals (the region of the AEP) with lower sound intensities diminishes and shifts to lower frequencies. In the pre-stimulus regions the amplitude and phase spectra always have irregular forms and lower amplitudes. Therefore, both the amplitude and the phase spectra are of importance in the objective detection of AEPs by Fourier analysis.

Further, the mean power ($P_{\bar{x}}$) of the post-stimulus averaged signal is higher than in a corresponding length of the pre-stimulus period (Table 1). This behaviour can be observed down to the hearing threshold in cases of low spontaneous EEG activity.

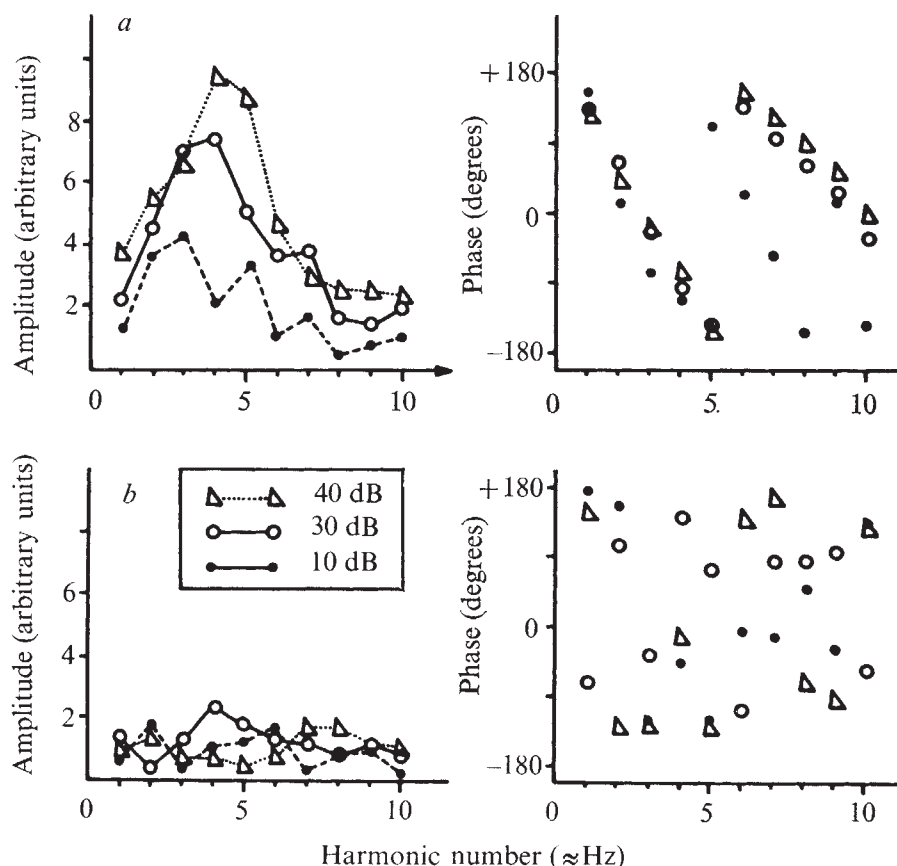


Fig. 1 Normal hearing female adult, binaural stimulation with 1-kHz tone bursts of 300-ms duration and 8-ms rise-fall time; interstimulus interval 3.2 s, $n = 50$ averaging cycles. Parameter: sensation level; frequency interval between harmonics 0.98 Hz; connecting lines illustrate typical behaviour. *a*, Amplitude and phase spectra of averaged responses (post-stimulus periods, 0-1,023 ms after onset of stimulation). *b*, Amplitude and phase spectra of averaged spontaneous activity (pre-stimulus periods, 1,800-777 ms before stimulation). Δ , 40 dB; $\circ$, 30 dB; $\bullet$, 10 dB.

To show that single AEPs consist of an additive contribution to the spontaneous EEG we evaluated the averaged power spectrum (by means of the sum of power spectra of the single EEG responses) for both pre- and post-stimulus periods. The mean power ($\bar{P}_{x_i}$) of pre- or post-stimulus electroencephalic activity can be calculated using this averaged power spectrum. The observed increase of $\bar{P}_{x_i}$ in the post-stimulus period (Table 1) is in agreement with the assumption of an additive superposition of the single AEPs to the spontaneous EEG (see ref. 3).

Averaging the squared single EEG responses, $x_i^2(t)$, gives the same result.

The evaluated mean square values

$$\overline{x^2(t)} = (1/n) \sum_{i=1}^n x_i^2(t)$$

are proportional to the mean power of the EEG. Because of an additive contribution of single responses to the spontaneous EEG an increase of the mean power in the region of the AEP is detectable down to 20 dB.

Using the mean square values $\overline{x^2(t)}$ it is possible to calculate the standard deviation $s(t)$ of the AEPs. In this way the statistical significance of registered AEPs can be evaluated without using the Fourier method³ with its more complex problems of utilisation.

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Table 1 Mean power of averaged potentials ($P_{\bar{x}}$) and mean power ($\bar{P}_{x_i}$) of electroencephalic activity*

1 kHz dB	$P_{\bar{x}}$ 0-511† (ms)	$P_{\bar{x}}$ 512-1,023‡ (ms)	$\bar{P}_{x_i}$ 0-511† (ms)	$\bar{P}_{x_i}$ 512-1,023‡ (ms)
10	101	22	70	50
30	398	18	77	55
40	600	17	88	58

*Arbitrary units, different for $P_{\bar{x}}$ and $\bar{P}_{x_i}$.

†Post-stimulus periods.

‡Pre-stimulus periods.

In interindividual comparisons the enhancement of $\bar{P}_{x_i}$ in the post-stimulus regions has been found down to 10 dB but statistically significant for dB values ≥ 30 dB (5% level of significance, *t*-test of compounded samples only.)

1 Sayers, B. McA., Beagley, H. A., and Henshall, W. R., *Nature*, 247, 481-483 (1974).

2 Sayers, B. McA., and Beagley, H. A., *Nature*, 251, 608 (1974).

3 Finkenzeller, P., *Kybernetik*, 6, 22-44 (1969).

SAYERS AND BEAGLEY REPLY—The power of an averaged sensory evoked EEG response (AER) can increase with supra-threshold stimulus level, as von Specht and Kevanishvili have recapitulated¹. As pointed out by us², it often does so. The observation that even when this effect was negligible both an evident AER and a clear aggregation of Fourier spectral phases with repeated stimuli could still be seen, led us to investigate the relevance and use of phase spectra—our conclusions relate solely to that point.

But we disagree with von Specht and Kevanishvili¹ on three points. First, power changes in the AER cannot be used to deny a phase constraint hypothesis or, more specifically, to argue against the dominant importance of phase effects in respect of pattern that our results suggest.

Second, the evidence for the dominant importance of phase lies not in the extent of any AER power changes but in the finding that imposing the appropriate phase spectrum (that is, that of a supra-threshold AER) on a length of unstimulated EEG, converts this into a pattern highly correlated with the true supra-threshold response. Repeating the experiment by imposing the amplitude spectrum has no such effect.

Third, we think it inadvisable to base the detection of a true response on an assessment of average power in the post-stimulus EEG, in spite of attempts³ to use that method in support of other procedures. In our experience, spontaneous EEG (especially in the young child, where the auditory evoked response test is of practical importance) is often neither low level nor free from substantial unpredict-

able power fluctuations. These affect both the power in the averaged response and the average power in the individual lengths of post-stimulus EEG, and so interfere with recognition of any possible response, especially near threshold. In spite of the attractive simplicity of a power method, we therefore abandoned it in favour of a pattern-detection method.

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

On the first two points, we wonder if von Specht and Kevanishvili have overlooked the effect of increased coherence (in the sense of pattern similarity, between different lengths of on-going EEG subjected to a phase constraint on major spectral components) that could be expected to follow from increased supra-threshold stimulus level. The amplitude of the resulting averaged post-stimulus EEG would increase substantially, as we can

demonstrate using von Specht and Kevanishvili's own results (Fig. 1). Figure 1 shows the result of averaging 30 records of pseudo-post-stimulus EEGs derived from a length of unstimulated EEG subjected to a phase constraint altered according to seven values of a parameter θ . This sets the extent ($\pm\theta^\circ$) of uniformly random phase scatter (added independently and individually for each harmonic and for each of the 30 records) around the mean phase spectrum imposed—in this case, that of the 40 dB-stimulated example shown by von Specht and Kevanishvili¹ (their Fig. 1a).

The amplitude spectrum of the spontaneous EEG was retained unaltered throughout. Maximum pattern coherence is achieved for $\theta = 0$, and minimum for $\theta = 180$. Increasing the synchronisation of phase between records at repeated trials ($\theta = 180, 150, \dots, 0$) in accordance with increased stimulus level thus greatly affects pseudo-AER magnitude and power, and is sufficient to account for the power increase in AER tabulated ($P_{\bar{x}}$). If different lengths of spontaneous EEG are used for each individual pseudo-response record, typical fluctuations of on-going EEG power can intrude. Some trials showed almost no increase in pseudo-AER power for θ -values as small as 80, because of fluctuations in on-going EEG. Others showed larger changes.

This explains our third point of disagreement, matches well our observations, and illustrates why we think that the small change in average power ($\bar{P}_{x_i}$) with stimulus level tabulated by von Specht and Kevanishvili¹ does not significantly support their view. Nevertheless, we do regard the additivity of an evoked response as a useful model; our suggestion is simply that it is increasingly less useful as stimulus threshold is approached, even if only because of the sporadic fluctuations of on-going EEG. Attention is drawn to the importance of phase-constraint effects which, once recognised, suggest other and more objective ways than averaging to detect an evoked response. The phase effects created by a successful stimulus are a property of signal patterns, and are not restricted to the ERA situation.

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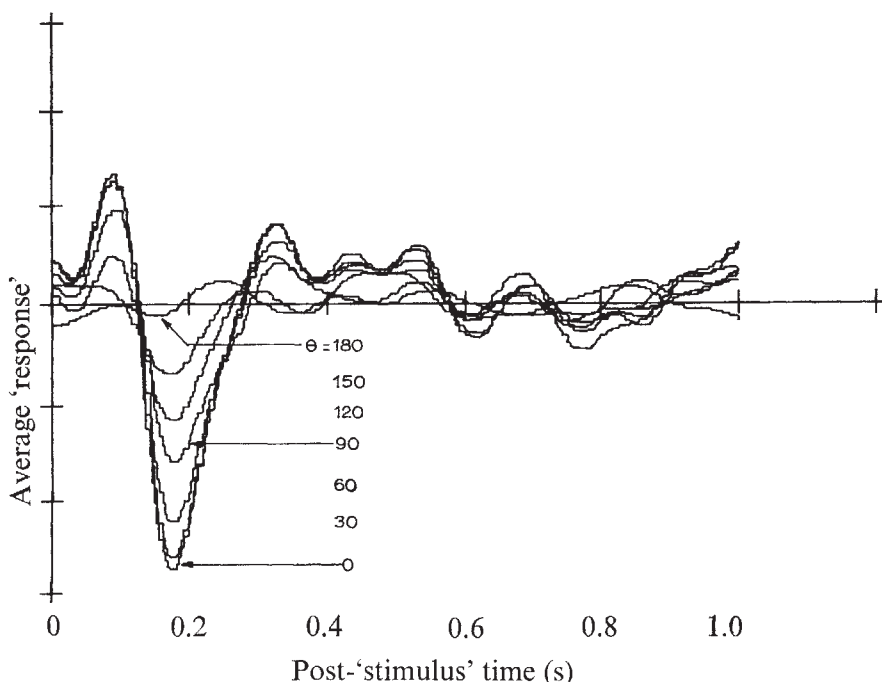


Fig. 1 Average of 30 records of pseudo-post-stimulus EEGs derived from a length of unstimulated EEGs subjected to a phase constraint altered according to seven values of the parameter θ (see text).

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¹ von Specht, H., and Kevanishvili, Z. Sh., *Nature*, 260, 461 (1976).

² Beagley, H. A., *Proc. R. Soc. Med.*, 65, 807 (1972).

³ Schimmel, H., Rapin, I., and Cohen, M. H., *Audiology*, 13, 33 (1974).

reviews

A (highly) intelligent school leaver about to take up an appointment as an X-ray crystallographer in a biochemical laboratory and wrecked on a desert island on his way, would find the present volume invaluable in equipping him for his new post by the time of his rescue. Dr Sherwood takes the reader in a methodical way through all the necessary steps to the solution of an unknown crystal structure. He starts with an explanation of the fundamentals of crystallography, of wave motion, of diffraction and Fourier transform theory and develops the necessary mathematical methods on the way. He then deals with the theory and practice of crystallographic structure determination—intensity measurement, extinction, Patterson methods, phase determination, refinement and direct methods are dealt with in successive chapters. The final section deals with biopolymers and diffraction by helical structures. In spite of the title, the chapter on protein crystallography as such is the least adequately covered. An appendix discusses a practical example of the solution of an

Desert island crystallography

U. W. Arndt

Crystals, X Rays and Proteins. By Dennis Sherwood. Pp. xxii+702. (Longman: London, January 1976.) £12.50.

organic structure.

The presentation is clear and fairly mathematical although it is a little doubtful whether it could be followed in its entirety by a reader who had not met any of the concepts before reading this volume. The author adheres throughout to a treatment in which he first states what he is going to say, then says it and finally discusses what he has said. The chapter summaries, in particular, are useful. Other good features are the annotated bibliography and the glossary and

index. References are mostly to monographs and reviews, rather than to original papers. The illustrations are plentiful and informative, although more modern X-ray photographs of biological materials might have been chosen. The text seems reasonably free of errors and misprints. It is a little strange to find within the same covers an illustration of wave interference in a ripple tank and a derivation of the Harker-Kaspar inequalities, but the author has fulfilled his stated intention of saying something of use for every class of reader in every section. Although the coverage of the theory of X-ray crystallography is fairly complete the reader will have to look elsewhere for a description of experimental methods.

This is a book to keep at home or in the office far from the departmental library, to read up on or verify X-ray crystallographic theory. It is of less value in a library since all the topics which the book covers can be found in readily available standard textbooks, although quite a number of separate ones may have to be consulted.

HAVING recently returned to academic life after several years in the oil industry, Dr Selley has obviously seen the need for a textbook on sedimentology with a distinct emphasis on the more practical and economic aspects of the subject, particularly in its application to petroleum geology. As indicated in the preface, the book presents an overview of the more important fields of sedimentology in an attempt to satisfy the needs and interests of students and practising geologists. It should of course be remembered that sedimentology has evolved rapidly over the past few decades, and presenting an overview must therefore necessarily involve concentrating on concepts rather than detailed analysis. Consequently, this book should be regarded strictly as a basic introduction to sedimentology in which subject matter is discussed at a very general level; but one which should also provide the reader with a reasonable background knowledge of the subject. The practising petroleum geologist may well have appreciated a more advanced treatment.

The text is presented according to a logical format, commencing with the now familiar summary of the textural characteristics of sediments, including

Do-it-yourself sedimentology

Brian Waugh

An Introduction to Sedimentology. By Richard C. Selley. Pp. xi+408. (Academic: London, New York and San Francisco, January 1976.) £5.90; \$14.75.

a valuable section on porosity and permeability. A short chapter on weathering is very disappointing, leaving the reader with little more than an elementary understanding of weathering and soil-forming processes. The sedimentary rocks themselves are covered in two chapters on allochthonous and autochthonous sediments, each major rock type being described with respect to classification, petrography and, where relevant, the relationship between diagenesis and porosity development. The sections on carbonates and evaporites are particularly useful summaries, although coverage of the other rock types suffers an inevitable brevity. Physical aspects

of transportation and sedimentation are qualitatively reviewed, followed logically by a discussion of the morphology and origin of biogenic and inorganic sedimentary structures. A major section is devoted to a study of sedimentary facies and environments, on the basis of which the author erects a series of informative sedimentary models. Sedimentary basins, their origin, structure and evolution are discussed, including a few short case histories illustrating the sequence and variety of basin fill deposits. The final chapter deals specifically with applied sedimentology, highlighting the role of sedimentological knowledge in the exploration and exploitation of hydrocarbons, together with a tantalisingly brief discussion of sedimentary ores.

The book is written in a very informal style, a feature which should appeal to the student reader at least, and the diagrams are on the whole clearly presented and informative. The major criticism of the book—that is, that too much has perhaps been attempted in this overview with the result that many topics are not discussed to any great depth—is perhaps counterbalanced, certainly for the more specialist reader, by the inclusion of most comprehensive reference lists.

Touching the right chord

Disease in a Minor Chord. By E. A. Steinhaus. Pp. xviii+488. (Ohio State University: Columbus, August 1975.) \$20.

THE late Edward Steinhaus has come to be regarded as perhaps the most significant 'founding father' of invertebrate pathology. This book was intended, in his own words, to be "semi-historical and semi-biographical", but was never finished because Steinhaus died in 1969. His widow, Mrs Mabry Steinhaus and one of his colleagues at Berkeley, Dr Mauro Martignoni, however, wisely did not attempt to complete the manuscript but instead concentrated on preparation for publication. The result is a book, presented with care and sympathy, which makes for fascinating reading.

Readers not familiar with this field may be surprised to learn of the struggle that Steinhaus had to obtain recognition for the importance and scientific merit of studies on diseases of insects and other invertebrates. Insects alone account for about 75% of the known animal species in the world and are man's greatest competitor for food as well as transmitting some of his most troublesome diseases. Nevertheless, the study of insect diseases remained an esoteric subject known to few men and scarcely at all to science until the late 1940s. It was typical of the period that when Steinhaus required a class text on insect pathology, none was available, so he had to write one himself.

Chapter 1 deals with the early history of insect pathology reaching into Greek science and ending in Chapter 2 with developments in immediate post World War II period. These two chapters will become prescribed reading for any student wishing to study the history of insect pathology. In Chapter 3 the significant contribution made by Californian workers is recounted. The prose in this chapter is perhaps the best in the book and it is the one in which we begin to know Steinhaus as the man rather than a familiar name in the literature. We can share his frustrations and also identify with his ambitions. Anyone who has tried to develop novel and possibly unconventional ideas cannot fail to have understanding, sympathy, but above all admiration for the task that Steinhaus set himself in California. We can rejoice that these hopes and aspirations were largely achieved, although not without pain and anguish. It is true that many major developments in insect pathology have appeared since 1969 but nevertheless it

must be acknowledged that the scene for these advancements was set largely by Steinhaus.

Chapter 4 presents an interesting, although inevitably incomplete, account of the European contribution to the knowledge of insect diseases, and in Chapter 5 we have Steinhaus' real and sometimes painful introduction to the intricacies of European scientific politics. Steinhaus the Editor was the creative force behind a scientific journal (*J. Insect Path.*, now *J. Invert. Path.*) and an annual review (*A. Rev. Ent.*) which have for many years now been accredited with international standing and the background story to these developments is given in Chapter 6.

It says much for the style and presentation of this book that it can be read and enjoyed by professional and layman alike and as such can be recommended to all. **T. W. Tinsley**

Fungal development

An Introduction to the Biochemistry of Fungal Development. By J. E. Smith and D. R. Berry, pp. 326 (Academic: London and New York, 1975.) £9.50; \$20.75.

ALTHOUGH few would deny that there are areas of molecular biology that require consolidation with further research, many molecular biologists (or are they microbial physiologists?) have turned to the comparatively virgin domain of microbial differentiation for a fresh challenge. Differentiation in micro-organisms is rather difficult to define, but fundamentally it entails the formation of morphologically different structures from vegetative organisms. Most microbiologists would go along with the great pioneer of research into biological differentiation, John Tyler Bonner, who believes that differentiation in organisms must be studied for the knowledge that it provides about those organisms. Nevertheless, many biologists who have turned to problems in microbial differentiation have done so because the system they have chosen to study provides a valuable model with which to study more complex metabolic differentiation processes that operate in higher animals and plants, the aim being to establish the basic mechanisms of control of gene expression as a cell switches from vegetative growth to formation of a differentiated structure. Not surprisingly, this upsurge of interest in microbial differentiation has generated texts and symposium proceedings, many of them excellent in their way although they are mostly

multi-authored volumes.

The main attraction of the text written by Smith and Berry, which restricts itself to differentiation in fungi, in which so many of the attractive systems are to be found, is that it is of a piece and does not suffer from the 'bittiness' that comes so often with a collection of articles written by different authors. The first of eight chapters deals with the conceptual basis of morphogenesis, and includes fundamental information on regulation of gene expression and enzyme action in cells. There follow two chapters on development in the slime moulds *Dictyostelium discoideum* and *Physarum polycephalum*, two very popular organisms for studying differentiation in multicellular systems. The fungal spore, the vegetative state, and asexual and sexual reproduction are the subjects of the chapters that follow, and the text concludes with a chapter entitled 'Differentiation, secondary metabolism and industrial mycology' describing production of compounds such as organic acids and antibiotics by moulds in what is essentially differentiation at the level of chemical synthesis as distinct from morphology.

The book has many attractive features. Each chapter ends with a brief summary, which will make it a valuable teaching text. Although at first sight there may seem to be too great an emphasis on fungal differentiation processes that have as yet been poorly researched, this is in many respects a richness for in this way the molecular biologist and biochemist can become acquainted with some of the more esoteric, but in differentiation terms very attractive, regions of mycology.

There are surprisingly few 1973 or 1974 references, which means that the book will quickly become dated. In general, Academic have done a fine job in producing the volume, although this was not helped by the authors electing to use line drawings of organisms rather than photomicrographs or electron micrographs, as well as some very old diagrams of life cycles. The text would have fulfilled its aim of providing an introduction to the biochemistry of fungal development if it had dealt in rather more detail with the molecular basis of regulation of gene expression and enzyme action. There is, for instance, no reference to specific degradation of enzymes or of enzyme inactivation by chemical modification such as adenylation.

The attractive features of the text, however, far outweigh these reservations, and it should prove a popular introduction for the teacher and for the researcher who wishes to enter this most challenging and fast developing area of biological research.

Anthony H. Rose

Surface physics not clarified

Surface Physics of Phosphors and Semiconductors. Edited by C. G. Scott and C. E. Reed. Pp. xiv+644. (Academic: London and New York, November 1975.) £18.80; \$48.75.

THE appearance within a few months of two important books which attempt to summarise surface physics suggests that this previously murky field is now beginning to take on a clearer shape. One book (M. Prutton's *Surface Physics*, recently reviewed in *Nature* (260, 374; 1976) is a compact organised teaching instrument which deals only with clean surfaces; the other, named above, aims to give a much fuller picture, and caters largely for solid-state device physicists in pursuit of better electronic and optoelectronic devices. It is essentially a series of research reviews which, although they do not overlap greatly, do not interlock too well either.

The first 300 pages contain introductions to the theory and method of surface physics. Our understanding of surfaces has come mainly from studying specially cleaned surfaces; the structure of these has been determined by electron diffraction and spectroscopy whereas theory has been developed by extending the wave mechanics of bulk materials. This section leaves one wondering whether the experiment and theory will ever be brought together to form a proper picture of the electronic nature of a surface state, even that found at a clean vacuum-solid interface, leaving aside the much more difficult problem of the 'real' interface which is formed beneath a native or intentionally-grown oxide film. It cannot be said that this book really grasps that nettle. All the important facts are there but the authors perhaps do not work hard enough at building them into a coherent whole. To quote an example from the editors' own 100-page review of the compound semiconductors: we are told about the striking increases in surface-state concentration in gallium arsenide which are produced by washing the surface with copper ions. Why should this happen? Although the previous 80 pages has described extensive investigations of the surface of compound semiconductors, none of the 'feel' which that might have provided is used to clothe the new, intriguing experimental fact with a few shreds of explanation. One chapter, however, does take the reader gently by the hand; this is Zaininger's highly original survey of some principles of device operation which may be particularly vulnerable to the presence of unwanted surface states.

Although the term 'phosphor' appears in the title, the physics of luminescence is hardly considered at all. The greatest phosphor, zinc sulphide, in fact gets one of the shortest treatments in the book. Few gaps, however, are left in the solid-state microelectronics field (possibly Schottky barriers and electron loss spectroscopy get less than their due) and workers in this field will gain from seeing this set of reviews in juxtaposition. It will also stimulate the thought processes of any physicist working on surface effects and may even spur one of the next generation to write the book which at last pulls together the theory and practice of the physics of semiconductor surfaces.

Andrew Holmes-Siedle

New mind, new body

New Mind, New Body: Bio-Feedback: New Directions for the Mind. By Barbara B. Brown. Pp.xiii+464. (Hodder and Stoughton: London, 1975.) £5.25.

THIS book is a well written, informative, popular introduction to biofeedback. Biofeedback is a method for training a person to increase his or her control over their own physiology. Barbara Brown effectively conveys the excitement and promise of this new field. *New Mind, New Body* is packed with information about biofeedback and about the way our mental and emotional selves interact with our bodily selves. To make her personal point (and this is one person's view of biofeedback) Dr Brown selects the evidence, but it's a good, thought-provoking selection. As a popular book, its style may be a little breezy and condescending for the specialist. The references are quite thorough and will be useful to the student and professional alike.

My personal fun was reading Dr Brown's rather caustic comments on the sterility of behavioristic psychology and its total inability to deal with the links between mind and body. She levels other criticism at mechanistic physiologists who have written of their speciality as if they had it in a jar completely isolated from the rest of the whole person. She points out what happened when behavioristic psychology mistook itself for the whole of liberal arts.

Dr Brown makes sure that her role in the "founding" of the Biofeedback Research Society is clearly documented in the early chapters. Researchers in the field knew it already but the public may want to know too. Succeeding chapters are each organised around

a particular physiological system. Within each chapter various biofeedback studies are discussed. This makes a readable sequence but it is not, I think, an organisation which identifies the major themes of biofeedback. For instance, mind-body and mind-brain mechanisms for learning, special effects of feedback, parameters of practice, parameters of the external feedback path, biofeedback as a system, are not discussed as salient topics.

The coverage of the bioelectric response of skin, musculature, heart, blood pressure and brain (recorded as a scalp EEG) is comprehensive. Yet, laced through the factual reporting are interpretations which range from expressions of indignation and sarcastic commentary, scientific judgments, to personal and rather woolly kinds of theories. Dr Brown really believes in mind as an object which can be studied by public science. She insists that biofeedback offers a method and that the results of biofeedback research gives a new basis for accepting the mind as a demonstrated phenomena. Her arguments are spirited, and her intentions are clear—she is not trying to pull her wool over the reader's eyes. But it is also a fact that many biofeedback researchers would make very different interpretations of the same data. For example, Dr Brown clearly believes that EEG phenomena can reveal a lot about the mind and that unique mind states are linked to unique EEGs. Yet, most users of EEG would find it difficult to defend the more conservative statement that unique brain states can be revealed in EEGs. The consensus is that the EEG doesn't provide a clue to subjective, mental life, and is a gross representation of the electrical activity of the brain.

The importance of biofeedback for training one's own physiological processes is undeniable and Dr Brown was one of the first to recognise the whole implication of this new technique. She is absolutely correct when she projects what can happen when and if humans learn how to control their own physiology by means of biofeedback.

The importance of biofeedback lies in its efficacy as a training method. Whether scientists know or agree on "how the person does it" is not really relevant to its practical value in laboratory and clinic. In fact the benefits of biofeedback are independent of the mind-brain, mind-body issue.

All-in-all it's a book worth reading. It's a personal journal produced by a lively person, with an informed mind and fearless spirit. As a popular exposition it's excellent and far ahead of its nearest rivals. **T. B. Mulholland**

Introducing mouse teratomas

Teratomas and Differentiation. (A Symposium held at the Roche Institute of Molecular Biology, Nutley, New Jersey, May 19–21, 1975.) Edited by M. I. Sherman and D. Solter. Pp. xv+324. Academic: New York and London, November 1975.) \$16.50; £8.60.

ALTHOUGH mouse teratomas have been available as an experimental system for more than a decade, it is only over the past few years that the application of immunological, cell culture and embryo micromanipulation techniques has begun to realise their enormous potential for studying regulatory mechanisms in mammalian development. It was in this climate of optimism (not without an element of elite evangelism) that a meeting was held at the Roche Institute of Molecular Biology in May, 1975 to consolidate and assess recent gains.

This book consists of the papers presented at the meeting, and is by far the best introduction to the subject at present available. It is a pity that the book does not also include discussion following the papers, along the lines of the CIBA symposia, so that the reader can enjoy the frisson of dissent and the benefits of a wider range of opinion. This would have been particularly useful following the papers by Barry Pierce and Beatrice Mintz in which, *inter alia*, it is argued that mouse teratocarcinomas are an important model for the study of cancer since they provide a case in which malignant stem cells give rise to benign progeny by differentiation. It is implied that many other cancers arise by a disruption in normal cellular interactions which reduce the probability that the progeny of stem cells will differentiate, and that this non-genetic malignant change should be reversible if only the correct environmental signals could be imposed. 'Malignant', however, is never clearly defined and the relevance of mouse teratocarcinomas, which seem rarely to metastasise or invade, to the corresponding human tumour, to non-malignant hyperplasia or to chemical carcinogenesis, is not discussed.

Murine teratocarcinomas arise spontaneously either in the gonads or from early embryos implanted into extrauterine sites. The comparative development of these tumours is clearly described by Leroy Stevens, who argues that the pluripotent stem cells are equivalent to the cells of the inner cell mass of the normal blastocyst. Evidence in favour of this view comes from the pioneering work of the

laboratories of Dorothea Bennett and Francois Jacob, which has shown that teratoma cells, sperm, morulae and blastocysts have in common a surface antigen which is probably coded for by one of the genes of the T locus. This work is the first step towards understanding the way in which early embryo cells may recognise each other by their surface properties.

Other exciting contributions are those of Gail Martin and Martin Evans on an *in vitro* method for obtaining relatively rapid and synchronous differentiation of homogeneous stem cells into endoderm, which in turn seems to trigger the next stages of embryogenesis, and the highlight of the meeting, which was the report by Beatrice Mintz that teratoma cells injected into normal embryos are able to participate in the formation of a normal, chimaeric mouse. The evidence for integration of teratoma cells into a wide variety of tissues, including germ cells of the testis, is impressive and the finding rich in biological implications. The book is worth reading for this paper alone.

Brigid Hogan

Iron transport and storage

Proteins of Iron Storage and Transport in Biochemistry and Medicine. (Proceedings of EMBO Workshop Conference on Proteins of Iron Storage and Transport.) Edited by R. R. Crichton. Pp. x+454. (North Holland: Amsterdam and Oxford; American Elsevier: New York, 1975.) Dfl.88; \$36.75.

THIS book contains the proceedings of the Workshop Conference on Proteins of Iron Storage and Transport held by the European Molecular Biology Organisation in Louvain-la-Neuve in April, 1975. The main themes presented in the book are the molecular biology of transferrin and ferritin, iron transport and storage proteins, respectively, and the subcellular transfer of iron by the intestinal mucosal cell during iron absorption. There is a total of 56 communications by contributors from Europe, the US and Australia.

In the section of the book devoted to transferrin all the outstanding problems related to its structure and function are brought to light and discussed. Two independent groups of workers presented almost the entire amino acid sequence of transferrin. Several papers deal with the interaction of the metal and anion at the binding site, and demonstrate how iron and a ligand (with a carboxylic group) fit snugly into

the receptor pocket on the protein surface. In addition, the distances between the iron, carbon and nitrogen (or the nitroxyl group) within the binding site have been measured using nuclear magnetic resonance and found to be about 1 nm. I find, however, that the most interesting investigations are not related to transferrin but to its specific receptor site on the surface of the immature red cell. The isolation and analysis of the transferrin receptor, a protein patch on the reticulocyte surface, initiated by Jack Fielding and Barbara Speyer, are bound to provide a solution to the biochemical basis of transferrin uptake and release by reticulocytes.

The contributions on the iron storage protein, ferritin, can be roughly divided according to their aim into the following groups. First, there are reports on the primary structure of this large molecule (molecular weight 450,000) composed of 24 subunits which form a shell enclosing a core of iron hydroxide and phosphate. In these reports the structure of subunits and their amino acid sequence are also described. Second, investigations on apoferritin subunit-subunit interaction and assembly are presented. These are followed by communications on identification of isoferritin in normal man and animals. The significance of isoferritins in malignant diseases is then discussed. Also, an attempt to determine the intracellular site of apoferritin synthesis and its turnover is described. Finally, but not least impressive, there is the work on iron uptake and release by ferritin carried out independently by Pauline Harrison *et al.* and by two groups of investigators led by Robert Crichton and S. Stefanini.

In the last section of the book several communications on the mechanism of iron handling by the intestinal mucosal cell during iron absorption are presented. The picture of subcellular iron pathways is not yet complete but intensive investigation under way in this field will no doubt bear fruitful results.

This book represents a faithful picture of the currents of research on iron metabolism carried out by investigators in many laboratories all over the world. The book is published within a few months of the Conference and thus it has a rare quality of freshness. The editor's skill in balancing finely the material presented makes it an exciting book to read. The reader with only a superficial interest in iron metabolism cannot easily appreciate the value of many hints so useful for bench work and may find many arguments too difficult to follow. For the iron enthusiast, however, this book is a 'must' and it certainly deserves space on a shelf of every reference library.

B. Brozovic

Most studied single cell

Biochemistry and Pharmacology of Platelets. (Ciba Foundation Symposium 35, New Series.) Pp. viii+352. (Elsevier/Excerpta Medica/North-Holland: Amsterdam, Oxford and New York, 1975.) \$23.95; Dfl. 59.50.

YET one more book on platelets! So prestigious a gathering as a Ciba Symposium, however, can be relied on to produce an authoritative book. What is the fascination that is fast making the platelet the most studied single cell? Partly, it is easily isolated yet more complex and interesting than the red cell: an understanding of it may contribute incalculably to the prevention of thrombosis. In addition, it is studied almost as a type cell. The actin and myosin are similar to that found in muscle. Dr Cohen exclaims "the amazing thing is that fibroblasts respond to exactly the same stimuli as platelets do". Biological amines and

monoamine oxidase drugs often have analogous effects on platelets and on the central nervous system. Another reason for the fascination is the close link of platelet biochemistry and pharmacology to platelet physiology. Indeed the contributors are as interested in pseudopod formation and sticky membranes as in the metabolic pathways involved: in fact large areas of platelet physiology are covered.

The basic membrane change involved in platelet 'stickiness' remains elusive but the membrane response to stimuli and the part played by the adenine nucleotides among many others, are presented in detail. The interrelationships between actin and myosin and the kinase of platelets and other cells are discussed. The pharmacology of platelet aggregation induction, its many inhibitors and the prostaglandins and aspirin effects are all well covered. The 'Platelet Release Reaction' has now become two: I from dense granules; II from α granules. Glycolysis, mineral elements, the part played by

calcium and the gangliosides—it is all here including receptor action and bioamine storage.

Although not wishing to detract from the interest of the many detailed studies presented, one questions the relevance of some to intact platelets *in vivo*. They are so sensitive that demonstrable alterations occur, for example, with a poor venepuncture or after a fatty meal. Although the effect of prostaglandins on the biochemistry is extensively considered, I would have liked more space devoted to the prostaglandin E_1 precursors and platelet inhibition—that elusive therapeutic goal.

As usual the standard of production is excellent. Professor Born and Ciba are to be congratulated, particularly on the editing of the discussion. But even so I don't find this nearly so rewarding as the papers themselves. This book is not easy going but is a recommended and masterly survey up to mid-1975 for physiologists and haematologists as well as biochemists and pharmacologists.

J. R. O'Brien

Learning theory

Elements of a Two-Process Theory of Learning. By J. A. Gray. Pp. x+423. (Academic: London, New York and San Francisco, 1975.) £6.60; \$16.50.

IN the past twenty years the study of animal learning has suffered a sharp loss in prestige; in the 1950s it was seen as the most developed and 'scientific' topic within experimental psychology; and great, although perhaps unrealistic, hopes were held out that it would illuminate our understanding of human behaviour and personality. These hopes have not been realised and, with the development of the cognitive and information-processing approaches to human behaviour, animal psychology is often seen as an unattractive and somewhat stagnant backwater with few connections with the wider currents of psychological thought. Dr Gray has argued that the study of animal learning cannot be divorced from human psychology, and that it has much to tell us about human personality. In this book he still sees learning theory as providing a "scaffolding for a theory of the physiological basis of personality".

It is a pleasure to read a book which conveys such enthusiasm for the subject particularly when it is written with style and clarity; it is by no means an elementary book and makes considerable demands on the reader, but no one who reads it could doubt that learning theory remains an interesting and challenging study, or that recent work on animal learning is providing

valuable information about behaviour.

In his attempt to provide a unified account of a wide range of animal behaviour, however, there is a strong tendency to rely on a small number of 'crucial' experiments to support a particular line of argument; in some cases this may be justified, but in others there are a variety of other experiments, which pass unreferenced, that do not support the author's point of view. For example, the evidence on the effects of superimposing an appetitive conditioned stimulus on instrumental behaviour maintained by an appetitive reinforcer is confused and conflicting; there is at least as much reason to suppose that it suppresses instrumental responding as that it increases it as Dr Gray maintains. Rescorla is cited as supporting the idea of backward conditioning, where the experiments in question were concerned with simultaneous not backward conditioning. These are not isolated examples, but rather illustrate a weakness present throughout the book; the unwary reader may easily be misled in such cases. The author also has a tendency to attempt to resolve issues by definition or logic. Thus it is argued that the word drive is used to describe three rather different concepts which are logically and operationally distinct; it is then claimed that in the light of these distinctions the classical questions about the role of drive reduction in reinforcement largely disappear. This logical coup, however, is achieved by making use of the concept of 'goal-

directed drive', which is foreign to most forms of learning theory, and which, although undefined, seems to subsume all the goal-directed aspects of motivation. The subsequent logical argument merely spells out the consequences of the author's definitional decisions and takes our understanding of motivation little further.

The whole book is an attempt to provide a unified account of learning which the author describes as a two-process theory. It is curious therefore that the theory proposed relies very heavily on the classical conditioning component and, as the author admits, is very similar to Mowrer's 1960 theory, which Mowrer himself described as a one-factor theory. In Gray's, as in Mowrer's, model the instrumental component serves only to direct the animal towards or away from stimuli which possess positive reinforcing properties; and in spite of the earlier discussion of 'goal-directed drive', the way in which goal directedness enters into these relationships is not discussed. What we seem to have is a series of taxes, rather than an integrated goal-directed response. In short, the author provides a plausible account of how stimuli becomes attractive or aversive, but very little insight about instrumental behaviour.

I feel that these shortcomings make the book unsuitable for undergraduate reading; but this is a book which research workers in the field will find sometimes illuminating, sometimes irritating but always interesting.

M. S. Halliday

obituary

Ben Dawes died at the age of 73 at his Burnley home on 3 March. He was formerly Professor of Parasitology at the University of London King's College, where he gave forty-two years of devoted service to the training of students and to research.

His first scientific appointment, after a hard-won start from being a weaver and then a tram cleaner, was to the Plymouth Marine Biological Station. Here he undertook research on plaice growth in relation to the availability of food, and his published papers on this continue to be standard references in fish feeding studies. In his early days at King's College he taught a wide spectrum of zoological subjects and in particular developed physiology and parasitology as final year undergraduate courses. His drive and enthusiasm were in no small measure responsible for establishing King's College as a centre of excellence in parasitology. His many students in this branch of study will remember his

stimulating lectures and his well organised practicals, with an array of excellently prepared material from all parts of the world. Perhaps, too, his post-graduate students will have cause to recall his insistence on accurate compilation of data, on precision in presenting and writing up results and also his considerable disdain for the use of scientific jargon.

Professor Dawes was recognised internationally for his investigations into many aspects of the biology and physiology of the sheep liver fluke, and of particular significance were his studies into the penetration of host tissues, migratory routes, feeding, growth and drug effects on the immature stages in mammals and in snails. All this work was carried out with infinite patience, dedication and meticulous care: he drove himself very hard. His world-wide status as a helminthologist was enhanced with the publication of his book on *'The Trematoda'*, first published in 1946,

reprinted in 1956, before being updated and re-issued in 1968. It still continues to stand the test of time as does his Ray Society Monograph on the trematodes of fishes. On assuming the editorship of the *'Advances in Parasitology'* series in 1963, Professor Dawes was insistent that it should reflect the progress in all areas of parasitology and the contents of the large number of volumes he edited demonstrate this resolve. He applied the same zeal, thoroughness and editorial criteria to each paper, irrespective of its specialisation, as he had to the work of his students, and always the advice and the criticism were intended to be helpful, a feature generally recognised by students and experienced workers alike. The 'cause' of parasitology was always near to his heart—a cause in which he lectured all over the world and in which he responded willingly to global requests for guidance and assistance. His demise leaves a gap in the ranks of world parasitologists.

announcements

Appointments

Dr Peter Ayscough has been awarded a personal professorship in Physical Chemistry at Leeds University.

Professor George F. Mitchell has been elected President of the Royal Irish Academy.

The Royal Society has elected the following as fellows: Professor Geoffrey Allen, Mr Sydney P. Smith, Professor Peter F. Baker, Dr Peter M. Biggs, Professor James Whyte Black, Professor Roger J. Blin-Stoyle, Professor Daniel J. Bradley, Professor John I. G. Cadogan, Professor William G. Chaloner, Professor H. Charnock, Professor Patricia H. Clarke, Professor Geoffrey Eglinton, Professor Roger J. Elliott, Dr Lloyd T. Evans, Professor Albrecht Fröhlich, Professor Frank W. E. Gibson, Dr Leonard G. Goodwin, Professor Edward G. Gray, Sir Ludwig Guttman, Dr John M. Hammersley, Sir William MacGregor Henderson, Dr Robin Holliday, Dr John H. Horlock, Dr George B. Mackaness, Dr Dan P. McKenzie, Professor John F. Nye, Dr Thomas G.

Pickavance, Professor Hubert Rees, Professor Joseph M. Ritchie, Mr Stephen J. Robinson, Professor Howard H. Rosenbrock, Dr John D. Smith, Professor Stanley D. Smith, Professor Francis G. A. Stone, Dr Brian A. Thrush, Sir Frederick E. Warner, Mr William Watt, Dr Michael J. Whelan, Dr Elsie M. Widdowson, Professor Ronald K. S. Wood.

Awards

Dr E. F. Hartree of the ARC Unit of Reproductive Physiology and Biochemistry, Cambridge, has been awarded the CIBA Medal and Prize for 1975.

The Zoological Society of London has awarded **The Scientific Medal** for 1975 to **Dr W. D. Hamilton**, Imperial College of Science and Technology for his work on evolutionary biology and to **Dr S. H. P. Maddrell**, University of Cambridge, for his work on secretory mechanisms in insects.

Professor Arthur Kornberg of the Department of Biochemistry, Stanford

University School of Medicine, has been chosen as the 1976 Jubilee Lecturer and recipient of the Jubilee Award of the Biochemical Society. Professor Kornberg has devoted much of his career to the study of enzymes involved in the replication of DNA. He was awarded the Nobel Prize in 1959.

The William Hopkins Prize of the Cambridge Philosophical Society has been awarded to **Dr S. W. Hawking** for his work on general relativity.

The Max Born Medal and Prize for 1976 has been awarded to **Hermann Haken**, Professor of Theoretical Physics of the University of Stuttgart for his work on the theory of lasers and also excited states in solids.

Academician **Ya B. Zeldovich** of the Space Research Institute Moscow has been awarded an Honorary D.Sc. by Sussex University. Amongst other topics, Dr Zeldovich has worked on Conserved Vector Currents, Relativistic Astrophysics, Shock Waves, High Temperature Hydrodynamics and Ultra-Cold Neutrons.

The Indian National Science Academy has announced the following awards for 1976: the Professor T. R. Seshadri 70th Birthday Medall to Professor R. C. Mehrotra of the University of Delhi, and the Silver Jubilee Commemoration Medal to Professor A. K. Sharma of the University of Calcutta.

International Meetings

May 12–13, **The Granulocyte: Function and Clinical Utilisation** (The Research Director, The American National Red Cross, Blood Research Laboratory, 9312 Old Georgetown Road, Bethesda, Maryland 20014).

May 19, **Scientific Research in Antarctica**, London (The Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG).

May 27, **The Biology of Chemical Carcinogenesis**, London (The Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG).

June 2–4, **Ozone Disinfection**, Chicago (Edward G. Fochtman, IIT Research Institute, 10 West 35th Street, Chicago, Illinois 60616).

June 4–5, **Isotope Effects on Enzyme-Catalysed Reactions**, Madison, Wisconsin (Dr W. W. Cleland, Dept of Biochemistry, University of Wisconsin, Madison, WI 53706).

June 6–9, **59th Canadian Chemical Conference**, London, Ontario (CIC, 151 Slater St, Ottawa, Ontario, Canada K1P 5H3).

June 6–13, **Zeolite '76**, Tucson, Arizona (F. A. Mumpton, Chairman Zeolite '76, Dept of Earth Sciences, State University College, Brockport, New York 14420).

June 7–8, **Biological Monitoring of Water Quality**, Helsinki (EIFAC Secretariat, Food and Agriculture Organisation of the United Nations, Via delle Terme, 00100 Rome, Italy).

June 8–10, **Trace Substances in Environmental Health**, Columbia, Missouri (Dr D. D. Hemphill, Chairman, 411 Clark Hall, University of Missouri-Columbia, Missouri 65201).

June 8–19, **COSPAR Meeting**, Philadelphia (Dean P. Kastel, Space Science Board, NRC, 2101 Constitution Av., Washington, D.C. 20418).

June 13–16, **Annual Meeting of the Society for Economic Botany**, Urbana, Illinois (Dr David S. Seigler, Dept. of Botany, 289 Morrill Hall, University of Illinois, Urbana, Illinois 61801).

June 21–23, **Mass Spectrometry in Drug Metabolism**, Milan (Dr A. Frigerio, Istituto di Ricerche Farmacologiche 'Mario Negre', Via Eritrea 62, 20157 Milan, Italy).

June 26, **The Early Years of the Edinburgh Medical School**, Edinburgh (A. D. C. Simpson, Royal Scottish Museum, Chambers Street, Edinburgh EH1 1JF).

June 29–July 1, **Tropical Conference on the Weak Interactions**, Falmer Sussex (Conference Secretary, Dr E. A. Sanderson, M.A.P.S., University of Sussex, Falmer, Brighton, Sussex BN1 9QH).

August 29–September 3, **Photobiology**, Rome (Deadline for abstracts: April 30) (Dr A. Castellani, Secretary-General, 7th International Congress on Photobiology, c/o Istituto Superiore di Sanita, Viale Regina Elena, 299, 00161-Rome, Italy).

September 7–10, **Meeting of the European Geophysical Society**, Amsterdam (Deadline for abstracts: June 15) (Mr C. R. Argent, EGS General Secretary, 6 Carlton House Terrace, London SW1Y 5AG, UK).

September 10–14, **Chapman Conference on Partial Melting in the Earth's Upper Mantle**, Southwestern Oregon (Deadline for abstracts: June 7) (AGU, 1909 K street, N.W., Washington, D.C. 20006).

September 27–29, **Pulmonary Macrophage and Epithelial Cells**, Richland, Washington (Deadline for abstracts: June 7) (Mrs. Judith A. Rising, Secretary to the 16th Annual Hanford Biology Symposium, Biology Dept., Battelle-Northwest, Richland, WA 99352).

October 21–23, **Annual meeting of the AGU Midwestern region-Eastern Section of the Seismological Society of America**, Ann Arbor, Michigan (Deadline for abstracts: July 27) (Meetings, AGU, Suite 1000, 1909 K street, N.W., Washington, D.C. 20006).

October 30–November 5, **1st Pan-American Congress and 2nd International Cinematographic Review of Orthopaedics and Traumatology**, Mexico City (Deadline: July 31) (Paseo de la Reforma No. 445, 4th floor, Mexico 5, D.F.).

November 22–26, **Radiobiological Research Needed for the Improvement of Radiotherapy**, Vienna (Deadline for Papers: June 1) (Dr B. B. Singh, Division of Life Sciences, International Atomic Energy Agency, Kärntner Ring

11, PO Box 590, A-1011 Vienna, Austria).

December 6–10, **Biological Applications of Stable Isotopes**, Leipzig (Deadline for papers: June 1) (Dr J. L. Servian, IAEA, P.O. Box 590, Kärntner Ring 11, A-1011 Vienna, Austria).

Reports and Publications

Great Britain

Proceedings of the Royal Irish Academy, Vol. 75, Section A, No. 14: a Joint Browder Essential Spectrum. By M. Snow. Pp. 129–131. 24p. Vol. 75, Section A, No. 15: Some Isoperimetric Bounds for the Torsional Rigidity of Anisotropic Elastic Cylinders. By J. N. Flavin. Pp. 133–144. 40p. (Dublin: Royal Irish Academy, 1975.) [112]

Molecular Aspects of Medicine, Vol. 1, No. 1, 1976. Edited by H. Baum and J. Gergely. Pp. 1–128 Annual subscription \$40. Vol. 1 will consist of six issues to be published bi-monthly. (Oxford and Elmsford, NY.: Pergamon Press, 1975.) [112]

Agricultural Systems, Vol. 1, No. 1, January 1976. Edited by C. R. W. Spedding. Pp. 1–83. Subscriptions: Four quarterly issues per volume, £15; \$34.50. (London: Applied Science Publishers, Ltd., 1975.) [112]

Institute of Food Science and Technology (UK). 1974/75 Report of Council, Council Committee Reports, Branch Committee Reports. Pp. 24. (Oxford, Surrey: Institute of Food Science and Technology, 1975.) [112]

Chemistry and Industry Buyers' Guide 1976: Chemicals/Manufacturing Plant/Laboratory Equipment/Services/Scientific Book Publishers. Pp. 86. (London: Society of Chemical Industry, 1975.) [212]

The British Library. *Scientific Research in British Universities and Colleges, 1974–75*. Vol. 1: Physical Sciences. Part 1 (Sections 1–36). Pp. xxii + 1–862. Part 2 (Name and Subject Indexes). Pp. xviii + 863 – 1146. £14.80 net (not sold separately). Vol. 2: Biological Sciences. Pp. xxii + 885. £11.25 net. Vol. 3: Social Sciences (Including Government Departments and Other Institutions). Pp. xxii + 844. £10.65 net. (London: HMSO, 1975.) [312]

Pricing for Pollution: An Analysis of Market Pricing and Government Regulation in Environment Consumption and Policy. By Wilfred Beckerman. Pp. 72. (London: The Institute of Economic Affairs, 1975.) £1. [312]

Department of the Environment. *Principles of Publication in Rescue Archaeology*. (Report by a Working Party of the Ancient Monuments Board for England Committee for Rescue Archaeology.) Pp. 15. (London: Department of the Environment, 2 Marsham Street, SW1, 1975.) [312]

Advice and Responsibility. By Lord Zuckerman. (The Romanes Lecture for 1975.) Pp. 40. (Oxford: Clarendon Press; London: Oxford University Press, 1975.) 95p net. [412]

Philosophical Transactions of the Royal Society of London: A: Mathematical and Physical Sciences. Vol. 280, No. 1295: Asymptotic Solutions of the Orr-Sommerfeld Equation. By J. M. De Villiers. Pp. 271–316. (London: The Royal Society, 1975.) UK £1.95; Overseas £2. [412]

Department of the Environment. *Community Land—Circular 1: General Introduction and Priorities*. (London: Department of the Environment, 2 Marsham Street, SW1, 1975.) 35p. [512]

Science Research Council. *Solid-State Devices: A strategy for SRC research support*. Pp. 17. (London: Science Research Council, 1975.) [512]

Science and Survival. (SISCON: Science in a Social Context.) By Ernest Braun and David Collingridge. Pp. 54. (Leeds: Siscon Project, University of Leeds, 1975.) [512]

University of Nottingham. *School of Agriculture Report 1974/1975*. Pp. 166. (Sutton Bonington, Loughborough, Leicestershire: University of Nottingham, School of Agriculture, 1975.) £1.20. [812]

The Royal Society. *Scientific Research in Schools Committee—Report to Council 1975*. Pp. 14. (London: The Royal Society, 1975.) [812]

Grasslands: An Intensive System of Lamb Production from Temporary Grassland. By N. E. Young and J. E. Newton. (Farmer's Booklet No. 1.) Pp. 28. (Hurley, Maidenhead: The Grassland Research Institute, 1975.) [812]

Memoirs of the Royal Astronomical Society. Vol. 80, Part 2: A Gravitational Three-Body Scattering Experiment. By M. J. Valtonen. Statistics of Three-Body Experiments—Probability of Escape and Capture. By M. J. Valtonen. Pp. 61–91. (Oxford and London: Blackwell Scientific Publications, 1975. Published for The Royal Astronomical Society.) [812]

Department of Employment. *Research 1974/1975*. Pp. iii + 70. (London: HMSO, 1975.) £1 net [912]

Tobacco Research Council. *Review of Activities, 1970/1974*. Pp. 114. (London: Tobacco Research Council, Glen House, Stag Place, SW1, 1975.) [912]

Department of the Environment. *Regional Strategy for the North West: Government Response to the Strategic Plan*. Pp. 15. (London: Department of the Environment, 2 Marsham Street, SW1, 1975.) [912]

Land, Air and Sea—Research in Man's Environment, 1965–1975. Pp. 48. (London: The Natural Environment Research Council, 1975.) [1012]

Cotton Research Corporation. *Cotton Research Reports, Republic of the Sudan, 1971/1972*. Pp. 26. 30p. Republic of the Sudan, 1972/1973. Pp. 32. 30p.

Swaziland, 1972/1973. Pp. 30p. Kenya, 1973/1974, Final Report. Pp. 61. 30p. Northern States, Nigeria, 1973/1974, Final Report. Pp. 61. 30p. Tanzania, 1973/1974, Final Report. Pp. 127. 30p. ULV Spraying for Cotton Pest Control. (Report on a workshop held at Lowveld Experiment Station, Big Bend, Swaziland, 3 to 7 March 1975.) Edited by N. Morton. Pp. 53. (London: Cotton Research Corporation, 14 Grosvenor Place, SW1, 1975.) [1012]

Science Research Council and Department of Industry. The Teaching Company: a Concerted Approach to Postgraduate Training and Advance in Manufacturing Engineering. Pp. 16. (London: Science Research Council, 1975.) [1112]

National Radiological Protection Board. NRPB-R36: Assessment of the Hazard to the Public from Anti-Static Brushes Containing Polonium-210 in the Form of Ceramic Microspheres. By G. A. M. Webb, B. T. Wilkins and A. D. Wrixon. Pp. 19. (Harwell, Didcot, Oxon: National Radiological Protection Board, 1975.) [1112]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 272, No. 920: The Morphology, Classification and Evolution of the Triceloidae (Trilobitidae). By C. P. Hughes, J. K. Ingham and R. Addison. Pp. 537-607 + plates 1-10. UK £4.05; Overseas £4.20. Vol. 273, No. 921: Petrology of Palaeosols and Other Terrestrial Sediments on Aldabra, Western Indian Ocean. By C. J. R. Braithwaite. Pp. 1-32 + plates 1-5. UK £2.55; Overseas £2.65. (London: The Royal Society, 1975.) [1112]

Agricultural Research Council. Annual Report of the Institute for Research on Animal Diseases for 1975. Pp. 66. (Compton, Near Newbury, Berkshire: Institute for Research on Animal Diseases, 1975.) [1512]

Ministry of Agriculture, Fisheries and Food. Food Additives and Contaminants Committee Review of the Lead in Food Regulations, 1961. Pp. 49. 65p net. Working Party on the Monitoring of Foodstuffs for Heavy Metals, Fifth Report. Survey of Lead in Food: First Supplementary Report. Pp. 34. 50p net. (London: HMSO, 1975.) [1512]

Imperial College of Science and Technology. Research Report, 1971/1974. Pp. 269. (London: Imperial College of Science and Technology University of London, 1975.) [1512]

Bulletin of the British Museum (Natural History). Zoology. Vol. 28, No. 8: A Revision of the Species of Lafoeidae and Haleciidae (Coelenterata: Hydrozoa). Recorded from Britain and Nearby Seas. By P. F. S. Cornelius. Pp. 373-426. (London: British Museum (Natural History), 1975.) £3.60. [1612]

1:250,000 Bouguer Gravity Anomaly Maps (Provisional Edition). Sheet 50°N-06°W. Land's End. (London: Institute of Geological Sciences, 5 Princes Gate, SW7, 1975.) [1612]

African Environment: Problems and Perspectives. Edited by Paul Richards, assisted by Nicola Harris. (African Environment Special Report 1.) Pp. xii + 118. (London: International African Institute, in association with the Environment Training Programme, UNEP-IDEP-SIDA, 210 High Holborn, WC1, 1975.) [1612]

Department of Industry, Technology and the Environment. (Reports from Scientific Counsellors Overseas, No. 9) Pp. 46. (London: Department of Industry, Abell House, SW1, 1975.) [1712]

Natural Environment Research Council. Annual Report of the Institute of Terrestrial Ecology for 1974. Pp. vii + 90 + 22 plates. (London: HMSO, 1975.) £2.75 net. [1712]

The University of Hull. Annual Report, 1974/1975. Pp. v + 106. (Hull: The University, 1975.) [1812]

Department of the Environment. The Welsh Office. River Quality and Discharges of Sewage and Industrial Effluents. (River Pollution Survey of England and Wales Updated 1973.) Pp. x + 62. (London: HMSO, 1975.) £2.60 net. [1812]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 273, No. 922: Observations on Growth and Morphogenesis in Cultured Cells of Carrot (*Daucus carota* L.). By F. C. Steward, H. W. Israel, R. L. Mott, H. J. Wilson and A. D. Krikorian. Pp. 33-53 + plates 1-8. UK £2.45; Overseas £2.55. Vol. 273, No. 923: Morphological Changes in the Thymus of Young and Adult Red-Billed Quelea *Quelea quelea* (Aves). By P. Ward and Marion D. Kendall. Histological Changes Associated with Enlargement and Regression of the Thymus Glands of the Red-Billed Quelea *Quelea quelea* L. (Procellariidae: Weaver Birds). By S. Bacchus and Marion D. Kendall. EMMA-4 Analysis of Iron in Cells of the Thymic Cortex of a Weaver Bird (*Quelea quelea*). By Marion D. Kendall. Pp. 55-82 + 6 plates. UK £2.50; Overseas £2.60. (London: The Royal Society, 1975.) [1912]

University of Oxford. Annual Reports, 1971-3. Pp. 52. (Oxford: The University, 1975.) £1. [2212]

Proceedings of the Royal Irish Academy. Vol. 75, Section B. No. 25: Lower Palaeozoic Rocks of the Shercock-Aghnamullen District, Counties Cavan and Monaghan. Pp. 499-530 + plates 11-16. £1.47. No. 26: Interrelationships Between Small Arthropods and Nematodes in Peat. By Ruth M. Blackith. Pp. 531-542. 25p. No. 27: The Chemical Composition of some Carbonate Secreting Marine Organisms from Connemara. By A. Gunatillaka. Pp. 543-556. 45p. (Dublin: Royal Irish Academy, 1975.) [2212]

The Radiological Centre. Medical Products: Clinical Diagnostics: Radiopharmaceuticals; Medical Radiation Sources, 1976/77. Pp. 103. (Amersham: The Radiochemical Centre, 1975.) [2212]

Report of the Welsh Plant Breeding Station for 1974. Pp. 146. (Aberystwyth: Welsh Plant Breeding Station, University College of Wales, 1975.) £1. [2412]

Finding and Identifying Mammals in Britain. By G. B. Corbet. (Publication No. 775.) Pp. 56. (London: British Museum (Natural History), 1975.) [2912]

Natural Environment Research Council. Report of

Person to person

NASA is continuing to encourage and support research on all aspects of lunar science and especially encourages well-qualified scientists not now in the lunar programmes to participate if they have new ideas, techniques, or research capabilities. An announcement giving details on where and how to propose may be obtained from Dr Edward A. Flinn, Deputy Director, Lunar and Planetary Programs, Code SL, National Aeronautics and Space Administration, Washington, DC 20546.

There will be no charge for this service. Send items (not more than 60 words) to Martin Goldman at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

the Institute for Marine Environmental Research, 1974/1975. Pp. 85. (Plymouth, Devon: Institute for Marine Environmental Research, 1975.) [3012]

Ministry of Overseas Development. Report on Research and Development 1975. Pp. v + 60. (London: HMSO, 1975.) £1.20 net. [3012]

Other countries

Smithsonian Contributions to Paleobiology, No. 25: Revised Tertiary Stratigraphy and Paleontology of the Western Beaver Divide, Fremont County, Wyoming. By Robert J. Emery. Pp. iii + 20. (Washington, DC: Smithsonian Institution Press, 1975. For sale by US Government Printing Office.) [2412]

United States Department of the Interior: Geological Survey. Professional Paper 924: Hurricane Agnes Rainfall and Floods, June-July 1972. (Report prepared jointly by the US Geological Survey and the National Oceanic and Atmospheric Administration.) Pp. vii + 403. Professional Paper 926-A: Geology and Resources of Base-Metal Vanadate Deposits. Pp. 14. Professional Paper 966: The Geologic Retrieval and Synthesis Program (GRASP). By Roger W. Bowen and Joseph Moses Botbol. Pp. iii + 87. (Washington, DC: US Government Printing Office, 1975.) [2412]

Royal Ontario Museum. Life Sciences Occasional Papers, No. 28: Karyotypes of Six Species of Bats (Chiroptera) from the Dominican Republic. By Donald W. Nagorsen and R. L. Peterson. Pp. 8. (Toronto: Royal Ontario Museum, 1975.) [1912]

United States Department of the Interior: Geological Survey. Professional Paper 926-B: Vanadium Resources in Titaniferous Magnetite Deposits. By R. P. Fischer. Pp. v + 10. (Washington, DC: Government Printing Office, 1975.) [3012]

Australia: Commonwealth Scientific and Industrial Research Organization. Division of Tribonphysics Report, 1973/1975. Pp. 35. (Melbourne: CSIRO, 1975.) [3112]

Transvaal Museum Memoir No. 19: A Review of Silk Production and Spinning Activities in Arthropoda with special reference to Spinning in Tenebrionid Larvae (Coleoptera). By L. Schulze. A Chromatographic Analysis of Tenebrionid Silk. By J. M. M. Brown. Pp. 51 + 24 plates. (Pretoria: Transvaal Museum, 1975.) [3112]

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Memoir 380 Quaternary Geology and Geomorphology, Nicola-Vernon Area, British Columbia. By Robert J. Fulton. Pp. xi + 50. \$8.40. Paper 74-36: Franklin Bay and Malloch Hill Map-Areas, District of Mackenzie. By C. J. Yorath, H. R. Balkwill and R. W. Klassen. Pp. vi + 35. \$4.20. Paper 74-53: Surficial Geology of Tuktoyaktuk, District of Mackenzie. By N. V. Rampton and M. Bouchard. Pp. v + 17. \$4.20. Paper 74-54: Geology of the Precambrian Ramah Group and Basement Rocks in the Nachvak Fiord-Saglek Fiord Area, North Labrador. By W. C. Morgan. Pp. vii + 42. \$3.60. (Ottawa: Information Canada, 1975.) [3112]

National Research Council Canada. Associate Committee on Scientific Criteria for Environmental Quality. NRCC No. 14102: Methoxychlor—Its Effects on Environmental Quality. By D. R. Gardner and J. R. Bailey. Pp. 164. (Ottawa: Publications, NRCC, 1975.) \$2. [3112]

United States Department of the Interior: Geological Survey. Bulletin 1405-A: Changes in Stratigraphic Nomenclature by the U.S. Geological Survey, 1974. By George V. Cohee and Wilna B. Wright. Pp. iii + 36. (Washington, DC: US Government Printing Office, 1975.) [51]

World Health Organization. Technical Report Series, No. 578: Radioimmunoassay of Hormones for Clinical

Trials of Fertility Regulating Agents in Developing Countries—Report of a WHO Meeting of Experts. Pp. 24. Sw. Fr. 5. Public Health Papers, No. 63: Schizophrenia—a Multinational Study. (A summary of the initial evaluation phase of the International Pilot Study of Schizophrenia.) Pp. 150. Sw. Fr. 16. Monograph Series, No. 55: Laboratory Techniques in Brucellosis. Second edition. Pp. 163. Sw. Fr. 35. (Geneva: WHO; London: HMSO, 1975.) [51]

Building Scientific Institutions in India: Saha and Bhabha. By Robert S. Anderson. (Occasional Paper Series, No. 11.) Pp. x + 121. (Montreal: Centre for Developing-Area Studies, McGill University, 1975.) \$3.50. [61]

Canada: Ministry of State, Science and Technology. Media Impact. Vol. 2: Science, Mass Media and the Public. By Orest Dubas and Lisa Martel. (Findings of a Study Group made in 1973/1974 sponsored by the Ministry of State for Science and Technology.) Pp. 393. (Ottawa: Information Canada, 1975.) \$6. [71]

Smithsonian Contributions to Zoology, No. 170: The Systematics, Postmarsupial Development, and Ecology of the Deep-Sea Family Neotanaidae (Crustacea: Tanaidacea). By Lion F. Gardiner. Pp. iv + 265. (Washington, DC: Smithsonian Institution Press, 1975. For sale by US Government Printing Office.) [81]

Atti del Tredicesimo Congresso Internazionale di Meteorologia Alpina/Proceedings of the Thirteenth International Meeting on Alpine Meteorology, Saint Vincent, Valle d'Aosta, Italia, 17-19, IX, 1974. Pp. 201. (Rivista Italiana di Geofisica e Scienze Affini, Vol. 1 (Speciale), Giugno, 1975.) (Genova: Società Italiana di Geofisica e Meteorologia, via Balbi 30, Casella Post, 3145, 1975.) [91]

Smithsonian Contributions to Zoology, No. 205: The Ecology and Behavior of a Subsocial Pentatomid Bug and Two Scutellid Wasps—Strategy and Counterstrategy in a Host and Its Parasites. By William G. Eberhard. Pp. iii + 39. (Washington, DC: Smithsonian Institution Press, 1975. For sale by US Government Printing Office.) [91]

United States Department of the Interior: Geological Survey. Bulletin 1313-E: Automatic Interpretation of Schlumberger Sounding Curves, Using Modified Dar Zarrouk Functions. By Adel A. R. Zohdy. Pp. iii + 39. Professional Paper 870-A: The Channels and Waters of the Upper Salmon River Area, Idaho. By William W. Emmett. Pp. viii + 116. (Washington, DC: US Government Printing Office, 1985.) [91]

Bibliography of Source Material On History Of Science and Technology In Medieval India: an Introduction. By Professor A. Rahman. Pp. 9. (New Delhi: National Commission for the Compilation of History of Sciences in India, Indian National Science Academy, 1975.) [121]

Reports of the Finnish Geodetic Institute. 75: 10: An Integrating, Centroid Timing, Receiver for Satellite Ranging. By A. B. Sharma. Pp. 23. 75: 11: A High Power Q-Switched Ruby Laser for Satellite Laser Ranging. By M. V. Paunonen. Pp. 8. (Helsinki: Finnish Geodetic Institute, 1975.) [121]

Australia: Commonwealth Scientific and Industrial Research Organization. Division of Soils Technical Paper No. 26: Contact Materials for Laboratory Soil Water Studies. By D. S. McIntyre and C. L. Watson. Pp. 11. (Melbourne: CSIRO, Division of Soils, 1975.) [121]

A Revision of the Australian Hylaeine Bees (Hymenoptera: Colletidae). By Terry F. Houston. (Australian Journal of Zoology, Supplementary Series, No. 36.) Pp. 135. (East Melbourne: Editorial and Publications Service, CSIRO, 1975.) [121]

Alberta Research Council. Report 75-7: Geology and Coal Reserves of the Ardley Coal Zone of Central Alberta. By M. E. Holter, J. R. Yurko and M. Chu. Pp. 41. (4 plates). (Edmonton: Alberta Research Council, 1975.) \$2. [121]

Recueil des Travaux du Centre Oceanologique de Bretagne (C.O.B.). Pp. 725. (Paris: Centre National pour l'Exploitation des Océans, CNEXO, 1975.) [121]

Smithsonian Contributions to Zoology, No. 208: Studies of the Subtribe Tachyina (Coleoptera: Carabidae: Bembidiini), Part III—Systematics, Phylogeny, and Zoogeography of the Genus *Tachyta* Kirby. By Terry L. Erwin. Pp. iii + 68. (Washington, DC: Smithsonian Institution Press, 1975. For sale by US Government Printing Office.) [121]

National Research Council Canada. Associate Committee on Scientific Criteria for Environmental Quality. NRCC No. 14104: Fenitrothion—The Effects of Its Use on Environmental Quality and Its Chemistry. Pp. 162. (Ottawa: Publication, NRC, 1975.) \$2. [151]

Canada. Department of the Environment—Fisheries and Marine Service. Miscellaneous Special Publication No. 27: A Practical Guide to the Anatomy and Physiology of Pacific Salmon. By Lynwood S. Smith and Gordon R. Bell. Pp. iv + 14. (Ottawa: Information Canada, 1975.) \$2.40. [151]

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Paper 74-40: Geology of the Lower Palaeozoic Formations in the Subsurface of the Fort Simpson Area, District of Mackenzie, N.W.T. By N. C. Meijer-Drees. Pp. v + 65. \$4.20. Paper 75-31: Borehole Geophysics Applied to Metallic Mineral Prospecting—a Review. By A. V. Dyck, P. J. Hood, J. A. Hunter, P. G. Killen, A. Overton, A. M. Jessop and A. S. Judge. Pp. 65. \$3. Index to Publications, 1959-1974. Pp. 138. \$4.20. (Ottawa: Information Canada, 1975.) [151]

Australia: Commonwealth Scientific and Industrial Research Organization. National Measurement Laboratory—Biennial Report, 1973/1975. Pp. 93. (Sydney: CSIRO, 1975.) [151]

Conodont Ultrastructure: The Subfamily Acanthodontinae. By C. R. Barnes and D. J. Slack. Pp. 21. (Life Science Contribution No. 106.) (Toronto: Royal Ontario Museum, 1975.) [161]